



Quality Assurance Project Plan

2009

Central Valley Bacteria Source Identification Study

Version 1

Contract Identification Number: UCD-RD-06-5-003, part of SJSURF to UCD Contract Account #22-1509-3932 (in support of SWRCB Master Contract #06-395-250)

July 2009

The Source ID Study is a coordinated monitoring effort between the University of California, Davis (UCD) and the Central Valley Regional Water Quality Control Board.



www.waterboards.ca.gov/swamp

A1, Element 1. Title and Approval Sheet

Program Title Central Valley Bacteria Source Identification Study

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Effective Date May 2009

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Project Manager: Stefan Wuertz

Signature: _____ Date: _____

QA Officer: Xunde Li

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Central Valley Regional Water Quality Control Board

Contract Manager: Catherine Gill

Signature: _____ Date: _____

QA Officer: Leticia Valadez

Signature: _____ Date: _____

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A3, Element 3. Distribution list

All project staff at the University of California, Davis (UCD) and all Central Valley Regional Water Quality Control Board (Central Valley Water Board) staff listed in the table below will receive copies of this Quality Assurance Project Plan (QAPP) and any approved revisions of this plan. The plan will be held at the UCD and will be available to any interested parties after final approval. The QAPP plan will be available to any interested party by requesting a copy from Rob Atwill and/or Xunde Li.

Table 1 Distribution List

Title:	Name (Affiliation):	Tel. No.:	No. of copies
Project Director	Rob Atwill (Population Health and Reproduction, UCDavis)	530 754 2154	1
Project Manager	Stefan Wuertz (Civil and Environmental Engineering, UCDavis)	530 754 6407	1
QA Officer (UC Davis)	Xunde Li (Population Health and Reproduction, UCDavis)	530 754 9752	1
Contract Manager	Catherine Gill (Central Valley Water Board)	916 464 4714	Original
QA Officer (Central Valley Water Board)	Leticia Valadez (Central Valley Water Board)	916 464 4634	1

A4, Element 4. Project/task organization

A coordinated monitoring study has been initiated by the Central Valley Water Board and UCD. The Regional Board has contracted with UCD to conduct laboratory analysis to include *E. coli* O157:H7 (O157) and *Bacteroidales*. The Central Valley Water Board will be responsible for collection of samples, to include field measurements, and analysis of total coliform and *E. coli* samples. Table 1 identifies all personnel involved with this study. Descriptions of each person's responsibilities follow the table. Figure 1 shows relationships between personnel.

Involved parties and roles.

Table 2 Personnel Responsibilities

Name	Organizational Affiliation	Title	Project Title/ Responsibility	Contact Information (Telephone number, fax number, email address.)
Rob Atwill	Population Health and Reproduction, UC Davis	Professor	Project Director	Tel: 530 754 2154 Fax: 530 752 7563 Email: ratwill@ucdavis.edu
Stefan Wuertz	Civil and Environmental Engineering, UC Davis	Professor	Project Manager	Tel: 530 754 6407 Email: swuertz@ucdavis.edu
Xunde Li	Population Health and Reproduction, UC Davis	Project Scientist	QA Officer	Tel: 530 754 9752 Fax: Email: xdli@ucdavis.edu
Catherine Gill	Central Valley Water Board	Environmental Scientist	Contract Manager	Tel: 916 464 4714 Fax: 916 464 4800 Email: cgill@waterboards.ca.gov
Leticia Valadez	Central Valley Water Board	Staff Chemist	QA Officer	Tel: 916 464 4634 Email: lvaladez@waterboards.ca.gov

Project Direct

The Project Director is Rob Atwill, who will provide supervision of all tasks and people related to the project for which the contractor is responsible. The Director will be responsible for various project audits at their discretion in order to ensure the Monitoring Plan and QAPP directives are met. Additionally, the Manager will be responsible for all contract management tasks, including: invoicing and reporting and oversight of project progress.

Project Manager

The Project Manager is Stefan Wuertz, who will be responsible for the analysis of *Bacteroidales* analysis and technical input to this study.

Quality Assurance Officer – UC Davis

The Quality Assurance Officer (QA Officer) for UC Davis is Xunde Li, who is the author of the QAPP and Monitoring Plan and will be responsible for the scientific integrity of the data collection effort throughout the duration of the study. The QA Officer is also responsible for technical dialogs with advisors and experts related to the study, and maintaining all original chain of custody and result forms. The UC Davis QA Officer works independently from the Project Director, Project Manager, and laboratory technicians.

Contract Manager

The Contract Manager is Catherine Gill. Responsibilities include maintaining a project file with all original documents related to decisions and documents, such as the original QAPP and Monitoring Plan; updating the official approved QAPP after a review of the evidence for change and with concurrence of the Project Director, Project Manager, and UC Davis QA Officer; and coordinating field and lab activities.

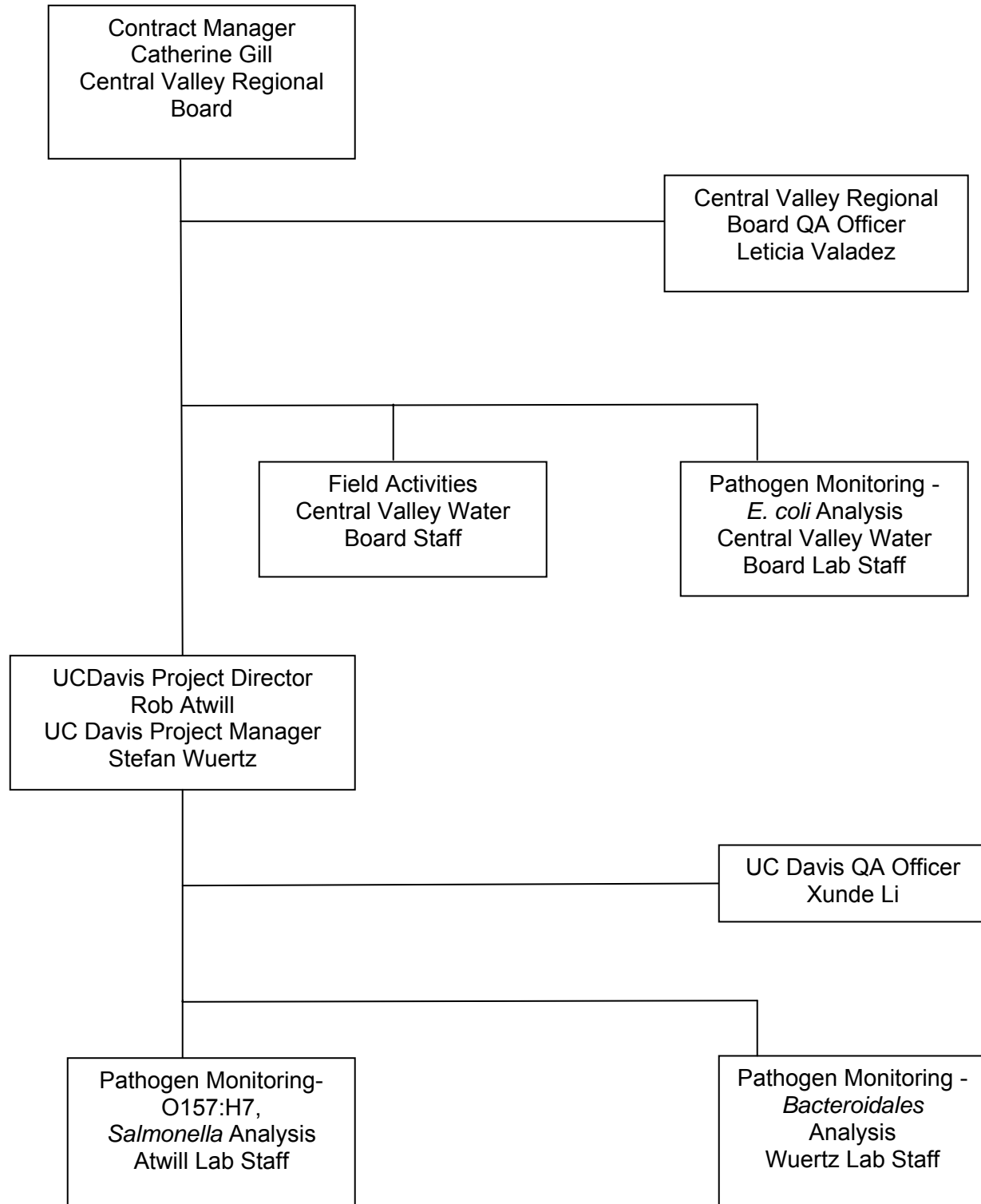
Quality Assurance Officer – Central Valley Water Board

The Quality Assurance Officer for the Central Valley Water Board is Leticia Valadez, who works independently from the Project Director, Project Manager, Contract Manager, field staff, and laboratory staff, and is responsible for the data meeting all quality objectives.

Field and Lab Personnel

Central Valley Water Board staff will provide the workforce for all field collection activities and analysis for *E. coli* samples. UCD laboratory personnel will provide the workforce for laboratory analyses and data management functions.

Figure 1. Organizational chart.



A5, Element 5. Problem Statement/Background

Problem statement.

Waterborne outbreaks of disease caused by microbial pathogen infection have been of increasing concern to public health. In 1997, the Legislature enacted AB 411 (Wayne, Chapter 765, Statutes of 1997), which required the California Department of Public Health (CDPH) to adopt minimum standards for testing of waters adjacent to public beaches for total coliform, fecal coliform, and enterococci bacteria, or other microbiological indicators, specifically along California's beaches. However, much of California's inland rivers and streams are also used for contact recreation. Recent O157 and *Salmonella* outbreaks between 1996 and 2006 brought attention to water supply systems and management practices used in raising crops. In 2007, a source identification study was conducted through the Central Valley Regional Board's Irrigated Lands Program in the lower San Joaquin River watershed, which utilized *Bacteroides* as the microbial source tracker. This study found that out of three categories (human, bovine, and chicken), the overwhelming majority of the *Bacteroides* DNA found in their samples was of human origin.

Long-term reduction of pathogen pollution will require an integrated approach that combines pathogen monitoring, microbial source tracking, and monitoring protocols that can detect trends in recovery or degradation of microbial water quality. The primary objectives of this 1-year project are to investigate the occurrence and pathogenic microbe, O157 in source watersheds of the Sacramento and San Joaquin Rivers and to identify possible vertebrate sources of fecal contamination. Data derived from this project will be used for developing strategies to remediate fecal contamination.

Decisions or outcomes.

This monitoring effort will provide water quality data within the Sacramento and San Joaquin River Watersheds to support SWAMP objectives. Specifically, this project will investigate the occurrence and source of pathogenic bacteria in waters from Sacramento River and San Joaquin River. Major questions being asked by this study are:

What are the spatial and temporal trends in bacteria indicator and pathogen concentrations at selected tributaries within the Sacramento and San Joaquin River Basins?

1. What are the potential sources of the identified bacteria (human, cow, dog, other)?
2. Is there any evidence that beneficial uses of recreation, drinking water, or irrigation water

supply are being impacted?

The primary objectives of this project are:

- Evaluate seasonal bacteria concentrations and trends in selected water bodies within the Central Valley of California
- Determine whether O157 is present at any time at any of the sites being evaluated
- Evaluate potential sources of fecal contamination and at a minimum group potential sources to human, cattle, or other
- Document viable vs. non-viable impacts
- Compare reported concentrations to appropriate water quality objectives and guidelines including but not limited to the Central Valley Regional Board Basin Plan (Basin Plan, 2007) and USEPA Bacterial Water Quality Standards for Recreational Waters guidelines (USEPA Standards, 2003).

Water quality or regulatory criteria

Staff at the Central Valley Regional Board initiated a water quality monitoring program in October of 2000 as part of California Assembly Bill AB 982 (Chapter 495, Statutes of 1999). AB 982 focuses State Water Resources Control Board (SWRCB) efforts to develop a comprehensive ambient surface water quality monitoring program known as the Surface Water Ambient Monitoring Program (SWAMP).

At the Central Valley Regional Board, SWAMP is attempting to answer the following overarching question and related sub-questions:

- Short Term:
 - What is/are the status and trends of ambient water quality in streams and rivers in the Sacramento River, San Joaquin River, and Tulare Lake Basins?
 - Are there spatial and temporal trends in water quality?
 - What is the location and extent of various levels of water quality?
 - Is there evidence of beneficial use impairment?
- Long-term:
 - Is water quality getting better or worse?
 - Are Board programs (regulatory/non-regulatory) and management actions effective?

During initial monitoring surveys conducted both by SWAMP and also the Irrigated Lands Regulatory Program (ILRP), elevated concentrations of E. coli were detected at numerous locations throughout both basins. In many instances, E. coli concentrations exceeded USEPA guideline of 235 MPN/100-ml for full contact recreation (swimming). Some sites exceeded the

guideline during every sampling event; other elevated concentrations appeared associated with flushing rainfall events. *E. coli* is an indicator of potential pathogen presence in a system.

A6, Element 6. Project/Task Description

Work statement and produced products.

This project will investigate the seasonal occurrence of *E. coli* and O157 in the source waters of the Sacramento and San Joaquin rivers and determine the source of fecal contamination through analysis of *Bacteroidales*. Sources will be grouped to human, bovine, dog and universal (all warm blood animals). In addition, field measurements, dissolved oxygen (DO), electrical conductivity (EC), pH, temperature, and turbidity, will also be analyzed.

The project will provide data sets after each sampling event, and fact sheets and a final project report at the end of the project.

Constituents to be monitored and measurement techniques.

Field measurements for DO, EC, pH, and temperature will be collected using a YSI 600XLM Sonde (Sonde) and 650MDS. Turbidity measurements will be collected using a portable Hach 2100P turbidimeter.

Laboratory measurements will include *E. coli*, O157, and *Bacteroidales*. Analysis for *Salmonella* may be conducted, depending on available resources.

E. coli analysis will be conducted by the Regional Board lab, utilizing the Idexx, Colilert-18.

At Atwill's laboratory, qualitative Enrichment-IMS method will be used for detection of O157. Membrane filters will be enriched in Tryptic Soy Broth followed by Immuno-magnetic separation (IMS). Specific PCR will be performed on positive samples to confirm the O157 serogroup and H-antigen designation.

At Wuertz's laboratory, source of fecal pollution will be determined through analysis of *Bacteroidales* using qualitative methods and quantitative models developed by the laboratory. At a minimum, sources will be grouped at least to human, bovine, dog and universal (all warm blood animals).

Project schedule

The project schedule outlined in Table 3 includes a pilot sampling run, which may be conducted prior to final approval of this QAPP to verify ability to meet logistical constraints with holding time, water volume, laboratory capacities, and transport requirements as specified in the associated QAPP. Should this run occur, sampling protocols will comply with the 2008 SWAMP Quality Assurance Management Plan (QAMP) for the State of California's Surface Water Ambient Monitoring Program (http://www.waterboards.ca.gov/water_issues/programs/swamp/qapp.shtml).

Table 3 Project Schedule Timeline

Activity	Date (MM/DD/YY)		Deliverable	Deliverable Due Date
	Anticipated Date of Initiation	Anticipated Date of Completion		
Start project	3/1/2009	5/31/2010	none	none
<i>Tentative logistical field run (Snowmelt event)</i>	<i>4/1/2009</i>	<i>5/31/2009</i>	<i>none</i>	<i>none</i>
<i>Tentative Sample Analysis (snowmelt event)</i>	<i>5/1/2009</i>	<i>7/31/2009</i>	<i>Data report</i>	<i>7/31/09</i>
Sample Collection (irrigation event)	7/1/2009	9/30/2009	none	None
Sample Analysis (irrigation event)	7/1/2009	10/31/2009	Data report	10/31/09
Sample Collection (dry season)	10/1/2009	11/30/2009	none	None
Sample Analysis (dry season)	10/1/2009	12/30/2009	Data report	12/30/09
Sample Collection (storm season)	12/1/2009	1/31/2010	none	None
Sample Analysis (storm season)	12/1/2009	2/28/2010	Data report	2/28/10
Draft report	2/28/2010	3/31/2010	Draft report to Water Board	3/31/2010

Activity	Date (MM/DD/YY)		Deliverable	Deliverable Due Date
	Anticipated Date of Initiation	Anticipated Date of Completion		
Final report	3/31/2010	5/31/2010	Final report to Water Board	5/31/2010

Geographical setting

The Sacramento River and San Joaquin River Basins cover about one fourth of the total area of the State and over 30 percent of the State's irrigable land. The Sacramento River Watershed is approximately 27,000 square miles and covers 17 percent of California's land. The San Joaquin Watershed covers 17,720 square miles. These watersheds consist of two major valleys, the Sacramento Valley to the north and San Joaquin Valley to the south. These valleys are bounded by several mountain ranges: the Coast Range to the west, the Cascade and Klamath Ranges to the north, and the Sierra Nevada Mountains to the east. The Sacramento watershed drains from northern California from the Oregon border to the Delta, where it joins the San Joaquin River and San Francisco Bay. The San Joaquin watershed originates in Madera County,

The Sacramento River is the largest river in the watershed, with an annual average stream flow volume of 22 million acre-feet. The river is also the longest in the State, extending over 327 miles. Major tributaries to the Sacramento River include the Feather, Yuba, American, and Pit Rivers. The main stem of the Sacramento River and most of its major tributaries have been developed for water storage, flood control, and power generation.

The San Joaquin River is the principal drainage artery of the San Joaquin Valley. Average annual surface runoff for the watershed is about 1.6 million acre-feet. Major tributaries to the San Joaquin River include the Cosumnes, Mokelumne, Calaveras, Stanislaus, Tuolumne and Merced Rivers, which primarily carry snowmelt. Flows from the west side of the river basin are dominated by agricultural return flows since west side streams are ephemeral and their downstream channels are used to transport agricultural return flows to the main channel.

Both basins provide a myriad of uses from their headwaters to discharge into the Sacramento-San Joaquin Delta, including timber production, grazing, recreation, fish habitat, drinking water supply and especially along the valley floor, agriculture. Combined, the two basins represent approximately 45% of the irrigated acreage in California.

Sixteen (16) sites in the Sacramento and San Joaquin watersheds were selected for sampling.

Sites were chosen to represent a variety of uses and concerns in each of the watersheds. Units within the Regional Board, including the Irrigated Lands Reporting Program and the Concentrated Animal Feeding Operations (CAFO), were queried for input in selecting sites. Sites will be sub-grouped into Agricultural/urban, Agricultural/rural, Restoration, Trend, and CAFO. See Appendixes 1 and 2 for list and description, and map of sampling sites. Additionally, an online map can be located at <http://maps.google.com/maps/ms?hl=en&ie=UTF8&msa=0&msid=116520518202884119741.000468dd851a54a9425e0&t=h&z=8>.

Constraints

Time is our primary constraint. This study was initiated with the goal of being able to sample during a twelve month period, across a variety of seasons to determine temporal changes in pathogen concentration and source patterns. Delays in finalizing the contract and funding for this study resulted in a reduced period for conducting sampling events. The study period is further reduced by the time necessary to run laboratory analysis for the source identification portion of this study, since six to eight weeks are required to process *Bacteroidales* samples.

Additionally, seasons and weather may impact water sampling and some sites may not have water in dry seasons and some other sites may not be accessible in storm season.

A7, Element 7. Quality Objectives and Criteria for Measurement Data

This section contains the measurement quality objectives of this study and includes analyses both in the field and in the laboratory. Data quality indicators for this study will consist of the following:

<u>Measurement or Analysis Type</u>	<u>Applicable Data Quality Objective</u>
Field Measurements (DO, EC, pH, Temp, turbidity)	Accuracy, Precision, Completeness
<i>E. coli</i> , <i>E. coli</i> O157:H7, <i>Bacteroidales</i> , <i>Salmonella</i>	Precision, Presence/Absence, Completeness

There are two types of quality objectives. Measurement Quality Objectives (MQOs) relate to the quality of the measurement itself (e.g. accuracy or precision). The Data Quality Objectives (DQOs) relate to the entire data set its ability to answer a study question (e.g. completeness or representativeness).

DQOs for the proposed project will be based on MQOs for the analytes listed in Tables 4. All monitoring data obtained will be SWAMP-comparable.

The MQOs for field and laboratory measurements are listed in Table 4. Because the method for pathogen analysis in this study does not require filtering, the MQOs listed in Table 4 differ from those listed in the SWAMP QAPrP. Details on the specific pathogens method and quality assurance procedures can be found in Appendices 3-16.

Additional MQOs for data acceptability, test conditions, water chemistry, and sample handling are listed in Appendix A of the SWAMP QAPrP.

MQOs for the equipment used to measure water temperature, dissolved oxygen, conductivity, pH, and turbidity in this project are detailed in Table 4. With proper calibration, the range, accuracy, and resolution of each instrument will meet the manufacturer's specifications and meet the MQOs for individual parameters. These parameters are detailed in 7.1 through 7.6.

Accuracy

Accuracy is the measurement of a sample of known concentration and comparing the known value against the measured value. Accuracy is measured by determining the percent recovery of known concentrations of analytes spiked into field sample or reagent water before extraction. The result of the calculation is evaluated against established acceptance limits.

Accuracy for bacteria will be determined by analyzing both positive and negative control samples. A positive control is similar to a standard. Positive O157 samples will be spiked with the target organism and then processed according to the procedures in Appendices 7, 8, and 9. Similarly, negative controls will be used to assess cross-contamination and the sterility of reagents and equipment.

Precision

Precision is a Data Quality Indicator (DQI) that measures the variability of repeated measurements of the same parameter in the same sample under the same analytical condition. Precision will be determined by having the same analyst complete the procedure for duplicate field samples (*E. coli* O157:H7) or laboratory duplicates (*Bacteroidales*). Our expectation is that duplicate samples will be ≥80% concordant (i.e., ≥80% agreement between pairs of samples). Two randomly chosen sites will have duplicate samples taken per sampling event.

Duplicate/recovery (precision) measurements are determined by field and laboratory replicates. The number of replicates for field measurements and samples taken will be 1 for every 10 sites (note that if there are 11 sites there will be 2 duplicates).

Comparability

Comparability is the degree to which data can be compared directly to similar studies. We will be using a method for *E. coli* O157:H7 and for microbial source tracking that have been peer-reviewed and published in the scientific literature (see Appendices 7, 8, 9, and 10).

Completeness

Completeness is the fraction of planned data that must be collected in order to fulfill the statistical criteria of the project. Volunteer data will not be used for legal or compliance uses. There are no statistical criteria that require a certain percentage of data. However, it is expected that 80% of all measurements could be taken when anticipated. This accounts for adverse weather conditions, safety concerns, and equipment problems. We will determine completeness by comparing the number of measurements we planned to collect compared to the number of measurements we actually collected that were also deemed valid. An invalid measurement would be one that does not meet the sampling method requirements and the data quality objectives. Completeness results will be checked for each sampling season. This will allow us to identify and correct problems.

Representativeness

Representativeness describes how relevant the data are to the actual environmental condition. Bias or lack of representativeness can occur if:

- Samples are taken in a stream reach that fails to describe the area of interest,
- Samples are collected in an unusual location, for example: a stagnant pool instead of the flowing portion of the water body,
- Samples are not preserved, stored, or analyzed appropriately, causing conditions in the sample to change, for example: bacteria samples not being analyzed within the 24 hour holding time from collection.

Representativeness and resulting bias are addressed through the overall sampling design. Sites were selected to identify potential contributors to fecal contamination and the sample schedule was designed to maximize representativeness by optimizing the sampling frequency and location. Sample collection specifications are described in Appendix 5.

Method Detection Limit and Sensitivity

The Method Detection Limit is the lowest possible concentration the instrument or equipment can detect. This is important to record because we can never determine that a pollutant was not present, only that we could not detect it. Sensitivity is the ability of the instrument to detect one concentration from the next. Detection Limits and Sensitivities for field and laboratory data are noted respectively in Table 4.

Table 4 Measurement Quality Objectives

Group	Parameter	Accuracy	Precision	Recovery/ Sensitivity	Target Reporting Limit	Calibration	Calibration Interval	Complete -ness
Field Testing (YSI 600XLM)	Dissolved Oxygen	± 0.5 mg/L	0.01 mg/L	NA	0.01 mg/L	Saturated air	Each sampling event	90%
Field Testing (YSI 600XLM)	pH	± 0.2 unit	0.01 unit	NA	NA	Buffer solutions pH 4, 7, and 10	Each sampling event	90%
Field Testing (YSI 600XLM)	Electrical Conductivity	$\pm 0.5\%$ of reading + 0.001 mS/cm	0.001 to 0.1 mS/cm (range dependent)	NA	0.001 μ S/cm	1000 μ S/cm standard	Each sampling event	90%
Field Testing (YSI 600XLM)	Water temperature	$\pm 0.15^{\circ}\text{C}$	0.01°C	NA	NA	Not required	Not required	90%
Field Testing (YSI 6920)	Turbidity	$\pm 2\%$ of reading or 0.3 NTU, whichever is greater	0.1 NTU	NA	0 to 1,000NTU	StablCal 2100P	Each sampling event	90%
Laboratory Analysis (Central Valley Regional Board)	Total coliform and E. coli	P/A	Lab duplicate, RPD < 25% RPD; Field duplicate within 95% CI supplied by Idexx (na if native concentration of either sample <	Field blank <1	1	NA	NA	90%

Group	Parameter	Accuracy	Precision	Recovery/ Sensitivity	Target Reporting Limit	Calibration	Calibration Interval	Complete -ness
			or > RL)					
Laboratory Analysis (Atwill Lab)	E. coli O157:H7	Positive and negative standards test ≥90% accurate	Duplicate samples ≥80% concordant	Distinguish 0 from ≥1 cfu	≥1 cfu per liter	NA	NA	80%
Laboratory Analysis (Atwill Lab)	Salmonella	Positive and negative standards test ≥90% accurate	Duplicate samples ≥80% concordant	Distinguish 0 from ≥1 cfu	≥1 cfu per liter	NA	NA	80%
Laboratory Analysis (Wuertz Lab)	Bacteroidales	Positive and negative standards test ≥90% accurate	Lab duplicates are ≥80% concordant	1-4 gene copies per PCR reaction per vertebrate source	1-4 gene copies per PCR reaction per vertebrate source	NA	NA	80%

A8, Element 8. Special Training Needs/Certification

Specialized training or certifications.

No specialized training or certifications are required for field staff for this project. The California Department of Health Services does not certify the Central Valley Water Board Lab, Atwill Lab, or Wuertz Lab.

All staff involved will be familiar with the field guidelines, fully trained in the aseptic technique of water sample collection and procedures. If necessary, additional training will be provided by Professors Atwill and Wuertz.

Field and lab staff will be required to review the SWAMP training manuals on field measurements and water sampling techniques, sample processing procedures, and the procedures outlined in Appendices 3 – 16 of this QAPP, as needed.

Training and certification documentation.

The Departments of Population Health and Reproduction and Civil and Environmental Engineering, UC Davis maintains training logs respectively for all personnel engaged in scientific research as is required by law. Those records can be obtained if needed from both laboratories.

Training records for the Central Valley Water Board staff are maintained at the Central Valley Water Board office. Laboratory safety manual and safety training records are maintained in the Central Valley Water Board's main lab.

All training records will be made available for review during audits.

Training personnel.

The Departments of Population Health and Reproduction and Civil and Environmental Engineering, UC Davis, provides training respectively to all personnel engaged in scientific research on an ongoing basis. Specific laboratory training is given to all personnel, including staff and students by the Laboratory Manager and Principal Investigators associated with the research in question. Laboratory safety trainings are provided by The Environmental Health and Safety Service of UC Davis and personnel training records are updated annually.

Central Valley Water Board staff are trained annually on field procedures and bacteria sample processing and analysis. Training is overseen by program managers who are responsible for their area of expertise.

A9, Element 9. Documents and Records

Documents and records generated from this project will be organized and stored in compliance with this QAPP. This will allow for future retrieval, and to specify the location and holding times of all records.

QAPP updates and distribution

All originals of the first and subsequent amended QAPPs will be held at the Central Valley Water Board office by the Contract Manager. The Contract Manager will be responsible for the distribution of the QAPP. Copies of the QAPP will be distributed to all parties involved with the project directly or by mail. Copies will be sent to the Atwill and Wuertz Laboratories, UC Davis directors/managers for distribution within the laboratory. Any future amended QAPPs will be held and distributed in the same fashion.

Records to be included in data reports

Data reports for each sampling event will include field data, laboratory data, chain of custody forms, sample processing forms, and associated QA reports.

All field data will be recorded at the time of completion, using the field data sheets (see Appendices 6 and 7).

Samples being sent to the Atwill and Wuertz Laboratories, UC Davis will include a Chain of Custody (CoC) form (Appendix 8). Laboratories will generate records for sample receipt and storage, analyses and reporting. The Data Manager will collate sample collection forms, sample transport forms, COC forms, as well as maintain the microbial database for O157:H7 and *Bacteroidales* results. All records will be delivered to the Regional Water Board Contract Manager, Catherine Gill, at project completion.

Additional documentation

Any documents not addressed in 9.2 that were generated in relation to this study will be submitted to the Contract Manager at the end of the study for insertion in the Source ID Study

project file.

Persons responsible for maintaining records

The Contract Manager will maintain the official project file, to include the original QAPP and Monitoring Plan, and subsequent revisions. The project file will also contain correspondence regarding decisions made, data reports, draft project report and final project report.

The official budget file will be maintained by Shalaka Joag at the Division of Sponsored Programs, San Jose State University Foundation, PO Box 720130, San Jose, California, 95172-0130.

Final disposition of records and documents

The project file will be stored in hard copy form at the Central Valley Water Board office for 3 years after completion of this project. After this time, files will be transferred to either Bekins or State Records Retention.

Copies of records at the Atwill and Wuertz Laboratories, UC Davis, will be maintained for a minimum of 5 years.

Electronic records

All data verified by the Contract Manager (field measurements, total coliform, and *E. coli*), Project Director (O157 and *Salmonella*), or Project Manager (*Bacteroidales*) will be forwarded electronically in Excel spreadsheets to the UCD QA Officer. Once all data is received, the UCD QA Officer will format all data and distribute data set reports, to include QA/QC information, to the Contract Manager, Project Director, and Project Manager.

The Contract Manager will store the data reports on the Central Valley Water Board network drive, which is backed up nightly.

B1, Element 10. Sampling Process Design

To address the problem statement in section 5.1, a sampling design was developed to generate

data that would provide input for the decisions and outcomes listed in section 5.2. The plan below outlines the proposed approach and is subject to modification as circumstances arise, such as drought or storm seasons. The project will measure field parameters, *E. coli*, O157 and *Bacteroidales*. (Additional samples may be analyzed for *Salmonella*, depending upon available resources.)

Description and Justification for Design Strategy

Sixteen (16) sampling sites were chosen throughout the Sacramento and San Joaquin River watersheds, to represent a variety of potential sources of pathogens to surface waters. Potential sources included urban/rural activities (wastewater), irrigated agriculture, grazing, and unknown sources. Site locations range from as far north as Red Bluff and as far south as Patterson. In the Sacramento Watershed, sampling sites in the farthest northern reaches stay close to Highways 5 and 45. In the San Joaquin Watershed, sampling sites spread from the Valley Floor to rural communities in the foothills.

Sites selected have a history of monitoring through the Irrigated Lands Reporting Program, Surface Water Ambient Monitoring Program, Department of Water Resources monitoring, or Concentrated Animal Feeding Operations. *E. coli* concentrations at these sites have ranged from <1 to >2420 MPN/100ml. The sample site list in Appendix 1 identifies the corresponding site number on the map in Appendix 2, the site description, watershed, monitoring programs previously conducting monitoring at each site, GPS coordinates, and additional notes to include potential sources of bacteria.

Sample timing and locations have been chosen to provide data to answer the objectives listed in section 5.2.

Type and Total Number of Samples

Samples collected at each site will consist of field measurements (DO, EC, pH, temperature, and turbidity), total coliform, *E. coli*, O157, and *Bacteroidales*. Additional samples for *Salmonella* analysis may also be collected, as resources become available. A total of 60 samples will be collected for total coliform, *E. coli*, O157, and *Bacteroidales*. Resources permitting, a maximum of 60 samples will be analyzed for *Salmonella*. This total includes quality assurance samples for field blanks, field replicates, and lab duplicates. Lab blanks will not be counted in the 60 total samples.

Table 5 Sample Frequency and Sites Sampled for Each Parameter

Parameter	Sample Frequency				Sites Sampled
	Spring Snowmelt	Irrigation	Dry	Winter Runoff	All
Field Parameters	X	X	X	X	X
Total coliform/ <i>E. coli</i>	X	X	X	X	X
<i>E. coli</i> O157:H7	X	X	X	X	X
<i>Bacteroidales</i>	X	X	X	X	X
<i>Salmonella</i> (as resources permit)	X	X	X	X	X

Analysis of samples will include enumeration of total coliform and *E. coli*, presence/absence of O157; and evaluation of *Bacteroidales*.

Sampling Location

GPS coordinates have been obtained from programs that have previously monitored at each sampling site.

To expedite locating sites, photo sheets of all the sites will be developed after the first sampling run for use in later runs. Photos will be taken with the image facing downstream of the sampling location.

Grab samples are to be collected at approximately mid-stream, mid-depth at the location of greatest flow (at least one inch below the surface) by direct submersion of the sample bottle. Whenever possible, samples will be taken away from the stream bank in the main current. Stagnant water will not be sampled. If it is necessary to wade into the water, the sample collector will stand downstream of the sample, taking a sample upstream. If the collector disturbs sediment when wading, the collector will wait until the effect of the disturbance is no longer present before taking the sample. Bottles will be rinsed three times on site with sample waters prior to sampling.

A description of each sampling site is included in Appendix 1.

Inaccessible Sample Sites

If sampling locations become inaccessible, field crews will look for an alternate site within 100 yards of the original sampling site. If no alternative site can be located, no sample will be collected.

Project Activity Schedule

Sampling events will take place within the sample collection periods listed in the project schedule in section 6.3. Field parameters will be measured on site. Samples to be analyzed in labs will be transported to the respective labs at the end of the field run. Sample analysis will begin within 24 hours of the time the first sample was collected.

Critical Information vs. Non-critical Information

All data results for *E. coli*, O157, and *Bacteroidales* are critical to reaching conclusions that will answer the objectives presented in A5, Element 5. Other information may also prove to be critical, such as weather patterns and field parameters. However, that determination cannot be made until the data is collected and evaluated.

Variability – Sources and Reconciliation

Natural variability occurs in any environment you will monitor. It includes seasonal changes in flow levels and source waters. One of the objectives of this study is to evaluate the seasonal and spatial variability in water quality within the study area.

Bias – Sources and Minimalization

Sample misrepresentation happens at the level of an individual sample or field measurement (e.g., collecting a water sample at a backwater pool that does not represent the bulk of the flow) and will be minimized by using SWAMP compliant training and sampling methods. Representativeness and bias are addressed in more detail in section 7.5.

B2, Element 11. Sampling Methods

Samples will be collected according to a combination of: a) Standard Operating Procedures as described in the SWAMP Quality Assurance Management Plan, Appendix 4, Field Protocols and b) Appendix E, SWAMP SOPs and recommended Methods for Field Data Measurements

and c) Standard Methods for the Examination of Water and Wastewater 20th Ed., which describe the appropriate sampling procedures for collecting samples for water chemistry and microbiology.

Field preparation

Field run preparation will consist of preparing field sheets (Appendices 6 and 7), chain of custody forms (Appendices 8 and 9), laboratory sheets (Appendices 9 and 10), sample labels, sample collection bottles, and verifying equipment functionality and availability.

Central Valley Water Board staff will be responsible for preparing all forms and obtaining sample bottles for O157, *Bacteroidales*, and *Salmonella* analysis from UC Davis.

On the day of the field run, prior to leaving the office, field personnel must sign out at the front desk and on the San Joaquin River Watershed Unit (SJRWU) sign out board located in the SJRWU section. Note your time of departure, time expected to return, and the mobile phone number.

Additionally, prior to leaving the office, samplers will need to complete the vehicle travel log and check the vehicle's tire pressure, oil, coolant, lights, etc. in order to avoid problems and/or delays while in the field. Report any major maintenance needed to the appropriate personnel and if needed retrieve an alternate truck.

Sample volume and bottle type

Field measurements will be taken on site and do not require a sample volume or collection bottle.

Samples collected for total coliform and *E. coli* analysis will be collected in either a 125 ml or 250 ml, factory sterilized and sealed polyethylene bottle. Sample volumes will be either 100 ml or 200 ml, respectively.

Samples collected for O157, *Bacteroidales*, and *Salmonella* analysis will each be collected in a sterilized 1 liter plastic bottle. Sample bottles will be filled to capacity. Bottles will be sterilized by UC Davis staff through autoclaving.

Sample bottle sterilization

Bottles used for collecting total coliform and *E. coli* samples are sterilized by the manufacturer and sealed.

Bottles used for O157, *Bacteroidales*, and *Salmonella* analysis will be sterilized by UC Davis. Verification that the bottles have been sterilized will be made by using autoclave tape, which changes color when the sterilization process is complete. The autoclave tape will be removed when samples are collected.

Sample preservation and holding times

Samples collected for total coliform and *E. coli* will be preserved with sodium thiosulphate which has been added to the bottles by the manufacturing company.

All samples to be analyzed in the lab will be preserved on ice at 6°C and transported in coolers (darkness) to the analytical labs at the end of the field run. The labs will process the samples within 24 hours after the first sample was collected.

Sample incubation times vary dependant on the analysis. Samples to be analyzed for total coliform and *E. coli* require incubation time of 18 to 22 hours. For consistency, samples will be pulled from the incubator at 18 hours and quantification run immediately. Samples to be analyzed for O157 require for 2 hours at 25°C followed by 8 hours at 42°C and those for *Salmonella* requires 24 hours at 37°C. No incubation time is required for *Bacteroidales* analysis.

Sample equipment

All sample collection items are located in the Central Valley Water Board office. Most items are stored in areas specifically designated for the SJRWU. The Calibration Room is a laboratory prep room located adjacent to the SJRWU lab, which is separate from the Central Valley Water Board's main lab. Sample collection equipment will consist of the following items:

Safety:

Equipment	Location
Roadside Emergency Kit	Garage SJRWU Locker
Toolbox	Garage SJRWU Locker
First Aid Kit	Garage SJRWU Locker
Jumper Cables	Garage SJRWU Locker
Rain Gear (as needed)	Garage SJRWU Locker
Floatation Vests (1 per sampler)	Garage SJRWU Locker
Cell Phone (charged)	Calibration Room

Field run requirements:

Equipment	Location
Ag/RB/SWAMP Field Books	Calibration Room
Clipboards w/field sheets	Calibration Room
pH/SC Kit	Calibration Room
Bucket (w/ Rope and Insect Repellant)	Garage SJRWU Locker
Potable Water	Garage SJRWU Shelf
Map Books (North + South)	Calibration Room
Shovel	Garage SJRWU Locker
SC Box	Garage SJRWU Shelf
Polyethylene 5-gal Bucket	Garage SJRWU Shelf
Vehicle Travel Pouch	Admin office (Reserved vehicle)

Sample collection:

Equipment	Location
Camera (charge battery)	Calibration Room
Sample Poles	Garage SJRWU Locker
Sample Coolers	Calibration Room
Ice	Garage Ice Machine
YSI MDS + Sonde	Calibration Room

All field equipment should be checked prior to each field event to verify functionality. In addition, the following procedures apply to selected equipment:

Field monitoring books: The field monitoring books contain summarized monitoring information (e.g. site location maps, contact information, etc.), access keys and emergency information for field personnel. Should there be any changes made to the monitoring schedules, the field monitoring books should be updated as well.

Field Sheets: Attach the field data sheet to the clipboard with a pencil, extra blank labels, and a photograph sheet of the monitoring sites.

Multipurpose meter/sonde: Retrieve the YSI multi-meter from the SJRWU Lab calibration room shelf. Record the identification number of the meter/sonde in the space provided on the field data sheet. Make sure the battery is fully charged and the DO membrane is good (i.e. with no bubbles) for the next day's use. Also include in the YSI bag a full service kit and a screwdriver. Included in the full service kit are:

- DO membranes
- Fine sanding disks
- O rings
- KCL Solution
- Grease
- Probe installation tool

- Tube brush
- Q tips
- Batteries

Tracking the YSI meters/sondes used in the field is important for proper maintenance. Calibration of the meter will be conducted the morning of sampling before leaving the office according to the instructions/cheat sheets (as detailed in the Procedures Manual for the San Joaquin River Water Quality Monitoring Program (Draft), July 2008) located in the calibration room cabinets. The YSI will also be checked or recalibrated at the end of the run and any time during the run if values seem not “normal.” These results are then recorded on the field data sheet in the space provided (Appendix 7).

A Myron L SC/pH meter should be included as a back-up for the field runs and stored in the pH/SC kit. Calibration of the meter should be conducted prior to use as a back-up. Calibration instructions for the Myron are on the back of the meter

pH/SC kit: The pH/SC kit is an ice chest containing items used for QA/QC during field sampling runs. Reorganize this cooler and augment any supplies as needed. The supplies include:

- SC calibration solution (3900µmhos) (1000 ml bottle)
- pH calibration solutions (4, 7, and 10) (1000 ml bottles)
- DI water (1000 ml bottle)
- Tap water (1000 ml bottle)
- Disposable nitrile gloves (small, medium, large, & extra large)
- Waterproof marker, ball-point pen, pencil, safety glasses, paper towels
- Myron L SC/pH meter
- Liquid soap
- Alcohol spray bottle w/extra alcohol
- Stainless steel cup

Sampling poles: Sampling poles are used to retrieve a sample up to six feet from the bank. Extensions are available and should be used as necessary. Sampling poles to be used to collect O157, *Bacteroidales*, and *Salmonella* are made from either PVC or stainless steel. Additionally, a stainless steel pole with a clamp attachment will be used to collect total coliform and *E. coli* samples.

Bucket and rope: A stainless steel bucket (triple rinse) and rope can be used for sampling off a bridge when there is no safe access to the bank or the distance is too great for the extensions. Additionally, the bucket can be used to carry sampling bottles to and from the site.

Cellular telephone: The SJRWU retains one cellular phone in the calibration room. While in the field, each sampling team must have a phone for emergencies.

Life vests: **Even the best swimmers can succumb to hypothermia.** The SJRWU has three life vests and additional life vests are available in the office. Life vests should be worn when sampling from bridges, boats, unstable banks, or during periods of extremely high flow.

Toolbox (blue) and road emergency box (red): Periodically inspect the toolbox and road emergency box to ensure that the appropriate contents are present and in working order. Contents are listed below:

Tool Box

Screwdriver, pliers
Flashlight
Graduated cylinder
Duct tape
Utility knife
Chain w/ locks
W-D 40
Sigma tubing
Toilet paper
Spare field keys
Spare C Batteries w/ YSI Cover
Utility Saw
Desiccant

Road Emergency Box

Flares
Reflector triangles
Fire extinguisher
First aid kit

Shovel/spades: Carry a shovel or spade in the truck in case the truck gets stuck and must be dug out.

Boots: Each sampler is responsible for being fit for their own rubber boots. If no boots fit the sampler, it will be necessary to have boots ordered. Hip waders can also be ordered as needed.

Ice: Prepare bags of ice the day prior to sampling and leave these bags inside the ice machine to be pulled by the sampling crew.

Water and food: Drinking water is necessary to maintain normal bodily functions. Bring plenty of water for a day. During the summer, double the amount of water you bring to avoid dehydration. While in the field, convenience food stores are not always available, therefore, pack a lunch and some snacks for the day. **Wash your hands prior to eating as we are sampling waterways that may contain unknown contaminants.** There are extra water coolers available to carry water for washing hands.

Personal protective gear: It is the responsibility of each sampler to think ahead and watch the weather forecast in order to dress appropriately for field monitoring. Rain gear is available in the SJRWU locker located in the garage area. Items that you may want to consider bringing from home are:

- Hiking boots
- Heavy coat/sweater/sweatshirt
- Hat
- Warm gloves
- Sunglasses
- Sunscreen

Other personal items: All field personnel will need a CRWQCB identification card that can be obtained from Donna Zupo, 916/464-4602. Carry this card in case you are asked to identify yourself by other agencies or the local growers. Money is also helpful for those unexpected necessities.

Double Check: After coolers with samples are placed out in the garage with the appropriate equipment, check all equipment off the checklist. The checklist is located in the garage on the SJRWU shelf.

Sample collection procedures

Sample collection procedures are summarized in Appendix 5.

Responsible person

The Contract Manager is ultimately responsible for coordinating field activities. However, staff may be delegated responsibilities for conducting the work to ensure all activities are completed. For instance, UC Davis staff will be responsible for ensuring that sample collection bottles for O157, *Bacteroidales*, and *Salmonella* analysis have been prepared. Also, Central Valley Water Board staff and/or students may be tasked to prepare field sheets, label sample bottles, and conduct sample collection field runs.

Any issues that cannot be readily corrected should be brought to the attention of the Contract Manager and noted on the field sheet. The Contract Manager will be responsible for investigating and resolving the issue.

B3, Element 12. Sample Handling and Custody

Maximum holding times

Field parameters do not have a holding time since the results will be determined on site. For all bacteria samples, the samples will be immediately placed on ice in a cooler for transport to the laboratories. All samples will be delivered at the end of the field run. Analysis will begin within 24 hours of the time the first sample was collected.

Maximum holding time stated in the Surface Water Ambient Monitoring Program Quality Assurance Program Plan (QAPrP) is 24 hours. The samples collected for this study are not intended as regulatory data.

Sample Handling

Identification information for each sample will be recorded on the label on the plastic sample bottles when the sample is collected. Sample identification is addressed below. Subsequently, identification information for each sample will be recorded on the laboratory data sheet (see Appendix 5) before submission to the Central Valley Water Board.

Samples received by the Central Valley Water Board lab will be processed immediately upon arrival. Samples received by the Atwill and Wuertz labs may be processed immediately, depending on arrival time in the respective labs. If logistics do not allow for immediate processing, the samples will be kept overnight in refrigerators in the respective labs to maintain a temperature of 6°C. All samples will be processed within 24 hours of sample collection.

Transport

Samples will be stored in coolers with ice, at a temperature of 6°C.

Samples to be analyzed for O157, *Bacteroidales*, and *Salmonella* will be delivered to
Atwill Laboratory
Room 2006, Haring Hall
UC Davis
One Shield Avenue
Davis, CA 95616
Tel: 530-754-9752

Samples to be analyzed for total coliform and *E. coli* will be delivered to
Central Valley Water Board Laboratory
11020 Sun Center Drive, #200
Rancho Cordova, CA 95670
Tel: 916-464-4799

Sample transfer

Field crews will deliver samples and required documentation to staff designated to receive samples. Samples collected will be verified against field sheets and chain of custody forms. Discrepancies and any additional notes, such as holding time exceedances, incorrect sample identification information, inappropriate sample handling, or missing/inadequate field equipment calibration information, will be noted on the field sheets and chain of custody forms, as needed.

by the staff receiving the samples.

Sample handling and custody documentation

All samples will be handled so as to minimize bulk loss, analyte loss, contamination or degradation. Sample containers will be clearly labeled. All caps and lids will be checked for tightness prior to transport. Samples will be placed in the ice chests with enough ice, or other packing, to include empty sample collection bottles, to completely fill the ice chest. Chain of custody forms will be placed in an envelope and taped to the top of the ice chest or they may be placed in a plastic bag and taped to the inside of the ice chest lid. Samples will be handled using aseptic technique so as to minimize chance for contamination.

Responsible individuals

The Contract Manager and Project Director will have ultimate responsibility for ensuring samples are properly handled and transferred. However, it is also the responsibility of the persons collecting, relinquishing, and receiving samples to initially verify correct sample handling and transfer.

Sample identification

Appendix 3 details the sample ID conventions, information that will be included on each bottle, and how samples will be labeled.

Chain of custody procedures

Field measurements do not require specific custody procedures since they will be conducted on site at the sample collection location.

All bacteria samples will be accompanied by chain of custody forms (Appendices 8 and 9). At the time samples are transferred, both the person receiving and relinquishing the samples should verify that all samples collected are reflected on the chain of custody forms. Any deviations should be explained on the field sheets and chain of custody forms, as needed.

B4, Element 13. Analytical Methods and Field Measurements

Field and laboratory analytical procedures

Table 6 Field Analytical Methods

Analyte	Project Action	Project Reporting	Analytical Method		Achievable Laboratory Limits	
	Limit (units, wet or dry weight)	Limit (units, wet or dry weight)	Analytical Method/ SOP	Modified for Method yes/no	MDLs (1)	Method (1)
Central Valley Regional Board						
DO	NA	NA	360.1	No	NA	NA
EC	NA	NA	b/120.1	No	NA	NA
pH	NA	NA	a/150.1	No	NA	NA
Temp	NA	NA	Temperature	No	NA	NA
Turbidity	NA	NA	*SM 2130B / EPA 180.1	No	NA	NA

(*) Standard Methods for the Examination of Water and Wastewater, 20th edition.

Table 7 Laboratory Analytical Methods

Table 1 Laboratory Analytical Methods						
Analyte	Project Action Limit (units, wet or dry weight)	Project Quantitation Limit (units, wet or dry weight)	Analytical Method		Achievable Laboratory Limits	
			Analytical Method	Modified for Method yes/no	MDLs	Method
Central Valley Regional Board						
Total Coliform	NA	NA	*SM 9221B, E	No	1 MPN/100 ml	NA
<i>E. coli</i>	NA	NA	*SM9221 B, +MUG	No	1 MPN/ 100 ml	NA
Atwill Laboratory						
E.coli O157	1 cfu/1000ml	1 cfu/1000 ml	Enrichment and ImmunoMagnetic Separation	None	Not applicable	Cooley et al., 2007
<i>Salmonella</i>	0.26 MPN/100ml	0.26 MPN/100	Multiple tube method	Yes	Not applicable	EPA method

		ml				1682
Wuertz Laboratory						
Bacteroidales	10 gene copies per mL	10 gene copies per mL	Rajal et al. 2007a	None	1 gene copy per reaction	Rajal et al. 2007a

(*) Standard Methods for the Examination of Water and Wastewater, 20th edition.

Laboratory Protocols

See Appendixes 11-16.

Instruments and kits to be used in field

Field measurements for DO, EC, pH, and temperature will be collected using the YSI 600XLM Sonde and 650 MDS. A probe guard will be attached at the end of the YSI to avoid fouling and protect the probes. If using the probe guard is not sufficient, the YSI may be hung by the bail from a sampling pole while the probe readings stabilize and are recorded.

Turbidity measurements will be collected using the Hach 2100P turbidimeter. Samples will be collected by using a stainless steel cup that is attached to a sampling pole and then poured into the glass vial. Care will be taken to minimize disturbance of the bottom of the stream bed. Should sediment be disturbed as a result of sample collection activities, the sampler will wait for the sediment to wash downstream before collecting the sample.

Equipment to be used for laboratory analysis

Equipment to be used for analysis of total coliform and *E. coli*:

Incubator – The primary unit used is Fisher Scientific Isotemp Forced Air Incubator, Model 650F. The backup incubator is the Binder® Incubator used by the CVRWQCB-Sacramento office lab.

Sealer – The Quantitray sealer is manufactured by Idexx.

UV Lamp – The UV lamp used to view samples positive for *E. coli* is Model EA-160 Spectroline ® 6-watt long wave ultraviolet lamp, which produces a wavelength of 365 nm.

Equipment to be used for analysis of O157:

Vacuum filtering system

Shaking incubator (Multitron II AJ120);

Dynal BeadRetriever (Invitrogen 159-50);
Fisher Scientific Isotemp Forced Air Incubator
Thermocycler for PCR
Electrophoresis device
Imaging System (Gel Logic 200)

Equipment to be used for analysis of *Bacteroidales*:

Vacuum filtering system
Real-time PCR System (combined thermocycler/light detection system)
Multichannel pipetters
Vortex Mixer with tube adapters

Equipment to be used for analysis of *Salmonella*:

Vacuum filtering system
Multichannel pipetters
Scientific Isotemp Forced Air Incubators

Method performance criteria

Unless otherwise noted, SWAMP reporting limits do not exist for constituents to be monitored in this study.

YSI 600 XLM Probes

(https://www.ysi.com/DocumentServer/DocumentServer?docID=EMS_S_XO)

Temperature

Sensor Type	Thermistor
Range	-5 to 50°C
Accuracy	±0.15 °C
Resolution	0.01°C
Depth	200 meters

Rapid Pulse Dissolved Oxygen, mg/L (Calculated from %air saturation, temperature and salinity)

Sensor Type	Rapid Pulse – Clark type, polarographic
Range	0 to 50 mg/L
Accuracy	0 to 20 mg/L, ±2% of the reading or 0.2 mg/L, whichever is greater 20 to 50 mg/L, ±6% of the reading

Resolution	0.01mg/L
Depth	200 meters

pH

Sensor Type	Glass combination electrode
Range	0 to 14 units
Accuracy	±0.2 units
Resolution	0.01 units
Temperature range	-5 to 50°C
Depth	200 meters

Conductivity

Sensor Type	4 electrode cell with autoranging
Range	0 to 100 mS/cm
SWAMP RL	2.5mS/cm
Accuracy	±0.5% of reading +0.001 mS/cm
Resolution	0.001 mS/cm to 0.1 mS/cm (range dependent)
Temperature Range	-5 to 60°C
Depth	200 meters

Hach 2100P Turbidimeter

(http://www.hach.com/fmmimghach?/CODE%3AL1619_09-0713836%7C1)

Method	EPA Method 180.1 (Nephelometric Ratio)
Range	0 to 1000 NTU
SWAMP RL	0.5 NTU
Accuracy	±2% of reading plus stray light
Precision	±1% of reading, or 0.01 NTU, whichever is greater
Resolution	0.01 on lowest range
Temperature Range	0 to 50°C
Depth	200 meters
Sample Required	15 mL
Sample Cells	60X25 mm borosilicate glass with screw caps

Total coliform and *E. coli* – Idexx Colilert 18 ®

Method	Standard Method 9221B, E (total coliform)
Method	Standard Method 9221B, +MUG (<i>E. coli</i>)
Sample size	100 mL
Shelf life	Up to 15 months at 2-25°C

Total coliform and *E. coli* - Idexx Quanti-Tray®/2000

Range	1 to 2419 MPN/100mL
SWAMP RL	2 MPN/100 mL

O157

Enrichment and ImmunoMagnetic Separation (see Appendices 11 and 12),

Method	Cooley et al., 2007
Reporting Limit	1 cfu/1000 mL

Bacteroidales

Method	Membrane filtration and Quantitative PCR (See Appendices 13 and 14), Rajal et al., 2007 a
Reporting Limit	10 gene copies per mL

Corrective actions

When failures in the laboratory occur, the Contract Manager, Project Director, and Project Manager will each be responsible for corrections in their respective laboratories. All failures will be documented on the field sheet and with the data report, along with the corrective action that was made. Additionally, corrections will be annotated in any applicable maintenance logs.

Sample disposal procedures

All of the UCD wastes resulting from culture, qPCR analyses will be handled as biohazard waste and discarded in accordance with Biohazard Waste Regulations and meets the requirements of UC Davis Environmental Health Service.

Samples analyzed for *E. coli* will be sealed in an Idexx Quantitrays. The Quantitrays will be delivered to the Sacramento Regional County Sanitation District at 8521 Laguna Station Road, Elk Grove, CA, to be autoclaved and disposed.

Laboratory turnaround times

Samples analyzed for total coliform and *E. coli* will have initial results within 24 hours of initiation of sample processing.

Samples analyzed for O157 will have initial results within 48 hours of initiation of sample processing.

Samples analyzed for *Bacteroidales* will have initial results between 6-8 weeks after initiation of sample processing.

Samples analyzed for *Salmonella* will have initial results within 4 days of initiation of sample processing.

B5, Element 14. Quality Control

This section describes the various laboratory and field quality control samples to be used in this study.

Quality control activities

The following checks will be utilized during each run to ensure quality control:

Table 8 Quality Control Checks

QC Check	Information Provided
BLANKS	
Laboratory blank	Assessment of background level of target analyte resulting from sample preparation and analysis
Field blank	Transport, storage, and field handling bias
CALIBRATION CHECK SAMPLES	
Zero check	Calibration drift and memory effect
Span check	Calibration drift and memory effect
Mid-range check	Calibration drift and memory effect
REPLICATES	
Field replicates	Precision of all steps after acquisition
Laboratory replicates	Analytical precision

Quality Control Samples

Quality control samples shall be collected according to a schedule pre-determined by UC Davis laboratories and the Central Valley Water Board. Specific quality control sample types are

described below.

Laboratory Blank

Laboratory blanks (also known as method blanks) provide bias information on possible contaminants for the entire laboratory analytical system. These samples will be made from phosphate buffered solution (for total coliform and *E. coli* samples) or sterile purified water (*E. coli* O157:H7 and *Bacteroidales* samples) that is known to have no detectable levels of the target analytes. Laboratory blanks will be analyzed along with the project samples to document background contamination of the analytical measurement system. The lab results must be less than the MDL of the target analytes to be acceptable.

Calibration Check Samples

Field measurement equipment will be checked for calibration against standards of known concentrations for pH and EC. Checks will be run at the beginning of the field run, after ten samples, and/or at the end of the field run.

Field Blank

Field blanks provide bias information for field handling, transport, and storage operations. They will be collected to evaluate whether contaminants have been introduced into the samples during sample collection due to exposure from ambient conditions or from the sampling containers. These blanks will be obtained by pouring phosphate buffered solution (PBS) into a sampling container at the sampling location. Field blanks will be preserved, packaged, and sealed exactly like the surface water samples and will be submitted blind to the lab. The lab results must be less than the MDL of the target analytes to be acceptable.

Field Replicates

Field replicate samples provide precision information on all steps after sample acquisition. These samples will be collected at designated sample locations by alternately filling two sample containers for each analysis. The field replicate samples will be preserved, packaged, and sealed in the same manner described for the surface water samples. A separate sample number and station number will be assigned to each duplicate and the samples will be submitted blind to the lab.

Field duplicates shall be collected immediately following the collection of normal samples. In

cases where multiple intermediate bottles are used for a single analysis, field duplicates and normal sample containers should be filled in an alternating sequence (i.e., normal-duplicate-normal-duplicate). Field duplicates should be submitted “blind” to the laboratories.

Field replicates should be submitted “blind” to the laboratory.

Laboratory Duplicates

Laboratory duplicates provide precision information on the analytical methods with the target analytes. The laboratory will generate the duplicate samples by splitting one sample into two parts, each of which will be analyzed separately.

Laboratory duplicate analyses should be requested for all constituents for the stations and events specified by the UC Davis Laboratory. No special sampling considerations are required. However, additional sample volume must be collected, per laboratory requirements, for each analysis.

Quality assurance frequency of analysis and measurement quality objectives

Quality control checks above will be conducted at the frequencies described below. Evaluation will be based on the measurement quality objectives listed in table 8:

Table 9 Quality assurance frequency of analysis and measurement quality objectives

LABORATORY QUALITY CONTROL	FREQUENCY OF ANALYSIS	MEASUREMENT QUALITY OBJECTIVE
Calibration Standard	Per analytical method or manufacturer's specifications	Per analytical method or manufacturer's specifications
Continuing Calibration Verification	Per 10 analytical runs	80-120% recovery**
Laboratory Blank	Per 20 samples or per analytical batch, whichever is more frequent	<RL for target analyte
Reference Material	Per 20 samples or per analytical batch, whichever is more frequent	80-120% recovery**
Laboratory Duplicate	Per 20 samples or per analytical batch, whichever is more frequent	RPD*<25% (N/A if native concentration of either sample <RL)

Internal Standard	Accompanying every analytical run as method appropriate	Per method
FIELD QUALITY CONTROL	FREQUENCY OF ANALYSIS	MEASUREMENT QUALITY OBJECTIVE
Field Duplicate	5% of total project sample count (3 samples total for this study)	RPD <25% (N/A if native concentration of either sample <RL; coliforms: within 95% confidence interval as defined by Idexx Laboratories)
Field Blank, Travel Blank, Equipment Blank	Per method	Blanks <RL for target analyte

$$*RPD \text{ (Relative Percent Difference)} = \frac{|(V_{\text{sample}} - V_{\text{duplicate}})|}{\text{mean}} \times 100$$

Where:

V_{sample} : the concentration of the original sample

$V_{\text{duplicate}}$: the concentration of the duplicate sample

Mean: the mean concentration of both samples

$$**\text{Reference materials and continuing calibration verification (\%recovery)} = \frac{V_{\text{analyzed}}}{V_{\text{certified}}} \times 100$$

Where:

V_{analyzed} : the analyzed concentration of the reference material or laboratory control sample (LCS)

$V_{\text{certified}}$: the certified concentration of the reference material or LCS

Corrective Actions

The Contract Manager is ultimately responsible for ensuring samples meet QA requirements and that appropriate corrective actions are followed. However, this does not exclude the UC Davis Project Direct, Project Manager, and QA Officer from maintaining responsibility for following QA/QC procedures for analyses conducted by UC Davis personnel.

The following table identifies QC samples and the corresponding corrective actions to be taken should problems arise.

Table 10 Corrective actions

Laboratory Quality Control (Bacteria)	Corrective Action
Calibration Standard	Affected samples and associated quality control must be reanalyzed following successful instrument recalibration
Initial/Continuing Calibration Verification	The analysis must be halted, the problem investigated, and the instrument recalibrated. All samples after the last calibration verification must be reanalyzed.
Laboratory Blank	The sample analysis must be halted, the source of the contamination investigated, the samples along with a new laboratory blank prepared and/or re-extracted, and the sample batch and fresh laboratory blank reanalyzed. If reanalysis is not possible, flag associated samples as estimated.
Reference Material	Affected samples and associated quality control must be reanalyzed following instrument recalibration.
Laboratory Duplicate	For duplicates with a heterogeneous matrix or ambient levels below the reporting limit, failed results may be qualified. Other failures should be reanalyzed as sample volume allows.
Internal Standard	As method requires. The instrument must be flushed with rinse blank. If, after flushing, the responses of the internal standards remain unacceptable, the analysis must be terminated and the cause of drift investigated.
Field Quality Control (Bacteria)	Corrective Action
Field Duplicate	For duplicates with a heterogeneous matrix or ambient levels below the reporting limit, failed results may be qualified. All failures should be communicated to the Project Director and/or Contract Manager, who in turn will follow the process detailed in the method.
Field Blank, Travel Blank	If contamination of the field blanks and associated samples is known or suspected, the laboratory should qualify the affected data, and notify the Project Director and/or Contract Manager, who in turn will follow the process detailed in the method.
Field Quality Control (Field measurements)	Corrective Action
DO, EC, pH, Temperature, Turbidity	The instrument should be recalibrated following its manufacturer's cleaning and maintenance procedures. If measurements continue to fail measurement quality objectives, affected data should not be reported and the instrument should be returned to the

Laboratory Quality Control (Bacteria)	Corrective Action
	manufacturer for maintenance. All troubleshooting and corrective actions should be recorded on the Central Valley Water Board field sheet (Appendix 7)

B6, Element 15. Instrument/Equipment Testing, Inspection, and Maintenance

Periodic maintenance

Field measurement equipment will be checked in accordance with the manufacturer's specifications. This includes battery checks and cleaning. All equipment will be inspected when first handed out and when returned from use for damage. Equipment will be maintained in accordance with its SOPs, which include those specified by the manufacturer and those specified by the method used in this study.

Field equipment inspection is carried out prior to each trip in the field. Testing is not conducted if equipment appears visibly worn or if field technicians report problems with the equipment upon returning from the field.

Maintenance/calibration logs are kept in the Atwill and Wuertz laboratories to log details including dates of instrument maintenance, calibration, and any problems noted. All necessary parts, reagents and calibration standards are kept on hand so that equipment can be kept in good repair and properly calibrated.

See table 11 Testing, Inspection, and Maintenance of Sampling Equipment and Analytical Instruments for list of all equipment to be used in this study and maintenance frequency.

Testing criteria

See table 11 Testing, Inspection, and Maintenance of Sampling Equipment and Analytical Instruments.

Persons responsible for testing, inspection and maintenance

The Contract Manager, Project Director, and Project Manager are responsible for ensuring

equipment relevant to their team is properly tested, inspected and maintained. Staff may be delegated the responsibilities of carrying out these tasks.

Spare parts

Location of spare parts for each piece of equipment is listed in Table 11 Testing, Inspection, and Maintenance of Sampling Equipment and Analytical Instruments.

Deficiencies

If deficiencies are found, the necessary maintenance will be performed and then the equipment will be re-calibrated and re-inspected. A pre- and post-calibration will be run to determine if the problem has been fixed. If this does not correct the problem, then the equipment will be taken out of use and sent to the manufacturer for servicing. Deficiencies that cannot be immediately corrected will be annotated on the field or lab worksheets, as applicable, and noted in the maintenance/calibration logs.

Table 11 Testing, Inspection, Maintenance of Sampling Equipment and Analytical Instruments

Group	Equipment / Instrument	Maintenance Activity, Testing Activity or Inspection Activity	Responsible Person	Testing/ Inspection Frequency	Location of Spare Parts	SOP Reference
Central Valley Water Board	pH probe (YSI)	Calibration Check	Contract Manager	Per field run	SJRWU Calibration Room	YSI User's Manual
	DO probe (YSI)	Calibration Check	Contract Manager	Per field run	SJRWU Calibration Room	YSI User's Manual
	EC probe (YSI)	Calibration Check	Contract Manager	Per field run	SJRWU Calibration Room	YSI User's Manual
	Temperature probe (YSI)	Inspection	Contract Manager	Per field run	SJRWU Calibration Room	YSI User's Manual
	Hach 2100P turbidimeter	Calibration Check	Contract Manager	Per field run	SJRWU Calibration Room	Hach User's Manual
	Incubator	Temperature check	Contract Manager	Per field run	Order as needed	Fisher Scientific, Binder User's Manuals
	UV Lamp	Inspection	Contract Manager	Per field run	Spare UV Lamp located in main Central Valley Water Board lab	Central Valley Water Board Bacteria Monitoring Procedures
	Sealer	Cleaning, Inspection	Contract Manager	Monthly	Order as needed	Idexx Sealer Manual
Atwill laboratory	Biosafety cabinet	Inspection	Project Director	annually	Room 2005 Haring Hall	The Baker Company's BSC instructions

Group	Equipment / Instrument	Maintenance Activity, Testing Activity or Inspection Activity	Responsible Person	Testing/ Inspection Frequency	Location of Spare Parts	SOP Reference
	Incubators	Temperature check	Project Director	daily	Room 2005 Haring Hall	VWR Incubator operating instructions
	Refrigerators	Temperature check	Project Director	daily	Room 2005 Haring Hall	REVCO manual
	Shaking incubators	Temperature check	Project Director	daily	Room 2005 Haring Hall	ATRBiottech instructions
	Pipettes	Calibration	Project Director	Every six months	Room 2005 Haring Hall	Eppendorf instructions
	UV Sterilizer	Cleaning	Project Director	As needed	Room 2005 Haring Hall	Millipore instructions
	Bead Retriever	Inspection	Project Director	annually	Room 2005 Haring Hall	DYNAL Invitrogen manual
	Thermocyclers	Positive and negative samples are included in all sample runs	Project Director	Every PCR event	Room 2005 Haring Hall	Eppendorf Mastercycler manual
Wuertz laboratory	Real-time PCR System (qPCR)	a) Background check, detector check b) Test internal fluorescence standard	Project Manager	a) Bi-weekly b) Every qPCR event	Room 2104 EU III	Operator manual ABI Prism 7000
	YSI handheld meters for conductivity meter	Inspected periodically throughout monitoring time period.	Project Manager	Weekly during water quality monitoring	Room 2104 EU III	Operator manual

Group	Equipment / Instrument	Maintenance Activity, Testing Activity or Inspection Activity	Responsible Person	Testing/ Inspection Frequency	Location of Spare Parts	SOP Reference
	(salinity), dissolved oxygen, temperature, pH			effort		

B7, Element 16. Instrument/Equipment Calibration and Frequency

All equipment and instruments are operated and calibrated according to the manufacturer's recommendations. Operation and calibration are performed by personnel properly trained in these procedures. Documentation of all calibration information is recorded in the appropriate logs. If equipment is not meeting the listed criteria (Table 12) it is the responsibility of the field crews and lab technicians to notify the Contract Manager, Project Director, Project Manager, and QA Officers, who will be responsible for addressing the problem. This may include repair or replacement of equipment. All corrective actions are documented in the appropriate log.

Table 12 Calibration of Sampling Equipment and Analytical Instruments

Group	Equipment / Instrument	SOP reference	Calibration Description and Criteria	Frequency of Calibration	Responsible Person
Central Valley Water Board	pH probe (YSI)	YSI User Manual	pH 7: ± 0.2 pH MV 4: +177 pH MV 7: ± 50 pH MV 10: -177	Per field run	Contract Manager
	DO probe (YSI)	YSI User Manual	Charge: 50 ± 25 Gain: -0.7 – 1.4	Per field run	Contract Manager
	EC probe (YSI)	YSI User Manual	Sp. Cond.: ± 20 Cal Const.: 5.0 ± 0.45	Per field run	Contract Manager
	Temperature probe (YSI)	YSI User Manual	YSI User manual	Per field run	Contract Manager
	Hach 2100P turbidimeter	Hach User Manual	$\pm 1\%$ of standard concentration	Per field run	Contract Manager
	Incubator	Fisher Scientific / Binder User Manuals	$\pm 0.5^\circ\text{C}$	Per field run	Contract Manager
	UV Lamp	Central Valley Water Board Bacteria Procedures	Positive Idexx comparator readings	Per field run	Contract Manager
	Sealer	Idexx User Manual	Tray Wells completely isolated	Per field run	Contract Manager
Atwill laboratory	Biosafety cabinet	The Baker Company's BSC instructions	As described in manual	annually	Project Director
	Incubators	VWR Incubator	As described in	routinely	Project Director

Group	Equipment / Instrument	SOP reference	Calibration Description and Criteria	Frequency of Calibration	Responsible Person
		operating instructions	manual		
	Refrigerators	REVCO manual	As described in manual	routinely	Project Director
	Shaking incubators	ATRBiotech instructions	As described in manual	routinely	Project Director
	Pipettes	Eppendorf instructions	As described in manual	annually	Project Director
	UV Sterilizer	Millipore instructions	As described in manual	routinely	Project Director
	Bead Retriever	DYNAL Invitrogen manual	As described in manual	annually	Project Director
	Thermocyclers	Eppendorf manual	As described in manual	annually	Project Director
Wuertz laboratory	Thermocyclers (PCR)	Wuertz Lab protocols Operator manual	Detectors: Part of regular maintenance performed by Applied Biosystems	Every run	Project Manager
	pH probe (YSI)	YSI User Manual (Model 63)	3-point calibration with technical buffers pH 7, 4, 10. Criteria: ± 0.2	Per each run	Project Manager
	DO probe (YSI)	YSI User Manual (Model 550A)	Calibration in % saturation	Per each run	Project Manager

Group	Equipment / Instrument	SOP reference	Calibration Description and Criteria	Frequency of Calibration	Responsible Person
			according to altitude and salinity		
	EC probe (YSI)	YSI User Manual (Model 63)	YSI conductivity standards. Sp. Cond.: ± 20	Per each water type or monthly (whatever comes first)	Project Manager
	Temperature probe (YSI)	YSI User Manual (Model 63 and 55A)	No calibration. Check with calibrated (DKD)/PTB) laboratory thermometers. Criteria $\pm 1^{\circ}\text{C}$	Quarterly	Project Manager

B8, Element 17. Inspection/Acceptance of Supplies and Consumables

Upon receipt and prior to use, all reagents will be inspected by the laboratory staff for broken seals and to compare the age of each reagent to the manufacturer's designated shelf life. Commercially prepared media for molecular biology analyses are used within the manufacturer's designated shelf life. All manufacturer-supplied specifications, which may include shelf life, storage conditions, sterility, performance checks, and date, are kept by the laboratories.

The Contract Manager, Project Director, Project Manager, and QA Officer are each responsible for inspection and acceptance of supplies and consumable used by their respective portions of this study. The actual inspection may be delegated to lab staff.

Details for each consumable are included in Table 13.

Table 13 Inspection/Acceptance Testing Requirements for Consumables and Supplies

Project-Related Supplies / Consumables*	Inspection / Testing Specifications	Acceptance Criteria	Frequency	Responsible Individual
Idexx Colilert Media Lot	Media properly reacts to known organisms when tested using the Idexx Quanti-cult	Organism (T. coli) (E.coli) E. coli (+) (+) K. pneumoniae (+) (-) P. aeruginosa (-) (-) Within expiration date	When new media lots are received	Contract Manager
Idexx Colilert Antifoam Solution	Contaminant free	Bottle tightly closed	Upon arrival	Contract Manager
Idexx Colilert Sample Vessels	Sterile Vessels	Vessels are sealed and accompanied with certification of sterilization	Upon arrival and prior to use	Contract Manager
Idexx Colilert Quanti-Tray/2000	Sterile Quanti-Tray/2000	Quanti-Tray/2000 are packaged in sealed bags and accompanied with certification of sterilization	Upon arrival and prior to use	Contract Manager
MoBio UltraClean™ Water DNA Isolation Kit (0.22 um)	Sterile Membranes	Membranes are sterile and sealed together with disposable filter	Upon arrival and prior to use	Project Manager
Tryptic Soy Broth	Organisms correctly enriched	Within expiration date	Upon arrival and prior to use	Quality officer
Dynal tips	Contaminant free	Sealed bags and accompanied with certification of sterilization	Upon arrival and prior to use	Quality officer
Dynal tubes	Contaminant free	Sealed bags and accompanied with certification	Upon arrival and prior to use	Quality officer

Project-Related Supplies / Consumables*	Inspection / Testing Specifications	Acceptance Criteria	Frequency	Responsible Individual
		of sterilization		
Anti- <i>E.coli</i> O157 DynalBeads	Properly reacts and attaches to <i>E.coli</i> O157	Correctly binds <i>E.coli</i> O157 Strain; within expiration date	Upon arrival	Quality officer
Rainbow agar	Media properly reacts to known organisms	<i>E.coli</i> O157 grown with proper morphology; within expiration date	Upon arrival and during use	Quality officer
CT-SMAC II agar	Media properly reacts to known organisms	<i>E.coli</i> O157 grown with proper morphology; within expiration date	Upon arrival and during use	Quality officer

*Additional items may include sterile pipettes, filter tips, PCR plates, PCR reagents, gloves.

B9, Element 18. Non-Direct Measurements (Existing Data)

Existing data

Multiple sample sites in this study are historic SWAMP, DWR, or ILRP stations (see Appendix 1). Data from these other sources will not be entered into the official SWAMP IMS through this project.

Data Quality Indicators (DQIs) will be used to judge whether the external data meets acceptance criteria. These include, for example, precision, accuracy, representativeness, comparability, completeness, bias, and sensitivity.

Measurement performance information such as method detection limits (MDLs), method quantification levels, and the selectivity of a method (or lack of sensitivity) for the target analytes will be used to judge whether the external data meets acceptance criteria.

Acceptance of external data for use will depend on the relevance of the matrix, location of the samples, and the methods that were used for collection and/or analysis (for example, field versus laboratory-based methods, the method of collection and analysis, etc.).

Water chemistry and field measurement data collected through SWAMP is available on the internet at http://www.waterboards.ca.gov/centralvalley/water_issues/water_quality_studies/surface_water_ambient_monitoring/sjr_swamp.shtml. Data collected by DWR is stored in DWR's Water Data Library, and data collected through the ILRP can be found at http://www.waterboards.ca.gov/centralvalley/water_issues/irrigated_lands/monitoring/index.shtml. Additionally, data collection stations for parameters such as flow and precipitation can be accessed through the California Data Exchange Center (<http://cdec.water.ca.gov>). Where appropriate, this data may be assessed in this study in order to better characterize long term trends.

Usage Limits

External data that fails to meet acceptance criteria will not be used in the project.

If and when external data does not meet acceptance criteria, it will, at the very least, be flagged as such. Flagged data may possibly be used under some conditions, but its use will be limited

and clearly designated.

B10, Element 19. Data Management

Data will be maintained as established in Element 9 above. All data from this study will be managed in accordance with the SWAMP data Management Plan (2009) and SWAMP Standard Operating Procedures (SOPs).

The Contract Manager maintains overall responsibility for proper data handling. Specific tasks may be delegated to other participants in this study. The Contract Manager will keep hard copies of monitoring related project documents in a dedicated binder. Monitoring related documents include: the Monitoring Plan (MP), the Quality Assurance Project Plan (QAPP), field logs, field data forms, COC forms and laboratory reports.

Data/information handling and storage

Recording, transcribing, digitizing, and downloading data

Central Valley Water Board staff will prepare field sheets (Appendices 6 and 7) prior to the field run to include sample run and sample location identification information. The sheets will be printed on waterproof paper – one per site of the UC Davis field data sheet, and one per field run of the Central Valley Regional Board field sheet.

Field crews will record observations and field measurement data at the sampling locations, using pencil. Prior to leaving the field site, field data sheets will be checked for completeness and accuracy.

Central Valley Water Board staff will record total coliform and *E. coli* analysis on the bacteria processing worksheet (Appendix 9). Data approved by the Contract Manager will be entered to an Excel worksheet and forwarded to the UC Davis QA Officer.

UC Davis staff will record O157, *Bacteroidales*, and *Salmonella* data on the laboratory datasheet (Appendix 10).

Bacteroidales raw qPCR data will be kept on the laptop that controls the Real-time PCR System and will be transferred to the Project Manager's computer for data analysis. Data approved by the Contract Manager will be entered to an Excel worksheet and forwarded to the UC Davis QA

Officer.

PCR raw data will be kept with the original software supplied by Applied Biosystems.

Transmittal

See A9, Element 9

Management

See A9, Element 9

Storage

See A9, Element 9.

Retrieval

The main contact for records will be through the Contract Manager, who will maintain the official project file. Contact information can be found in Table 2, A4, Element 4.

Computerized information system maintenance

Official electronic files will be maintained by the Contract Manager once the data reports are received from the UCD QA Officer. The file will be located on the Central Valley Water Board network at W:\nps\San Joaquin River\SJRWU-Special Studies\Bacti Source ID Study.

The Central Valley Water Board Information Technology unit performs backup nightly on all network drives.

SWAMP Information Management System

Field measurement, total coliform, and *E. coli* data will be verified as meeting QA/QC requirements by the Contract Manager. Once the data is verified acceptable, it will be entered into the SWAMP database by staff designated by the Contract Manager.

The SWAMP contract laboratories will submit the remaining data in SWAMP-comparable format to the SWAMP data Management Team for entry to the database.

Data in the SWAMP Database will be made available to the public through the California Environmental Data Exchange Network (CEDEN). CEDEN is currently in development and is expected to be operational in 2009. Information on CEDEN is available at www.ceden.org.

C1, Element 20. Assessments & Response Actions

Assessment and oversight involves both field and laboratory activities to ensure that the QA Project Plan is being implemented as planned and that the project activities are on track. By implementing proper assessment and oversight, finding critical problems toward the end of the project is minimized, when it may be too late to apply corrections to remedy them.

Two types of assessments may be used in this project: field assessments and laboratory assessments.

Field assessments will include:

- Readiness reviews to verify field teams are properly prepared prior to starting field activities;
- Field activity audits to assess field team activities during their execution; and
- Post sampling event reviews to assess field sampling and measurement activities methodologies and documentation at the end of all events or a selected event.

Laboratory assessments may involve two types of activities:

- Data reviews of each data package submitted by a laboratory; and
- Audits of laboratory practices and methodologies.

Project assessments

Readiness reviews will be conducted prior to each sampling run by the Contract Manager. All sampling personnel will be given a brief review of the goals and objectives of the sampling event and the sampling procedures and equipment that will be used to achieve them. Readiness reviews will consist of the following activities:

- Equipment checks – It is important that all field equipment be clean and ready to use when it is needed. Therefore, prior to using all sampling and/or field measurement equipment, each piece of equipment will be checked to make sure that it is in proper working order.

- Equipment maintenance records – Equipment maintenance records will be checked to ensure that all field instruments have been properly maintained and that they are ready for use.
- Supply checks – Adequate supplies of all items in B2, Element 11 will be checked before each field event to make sure that there are sufficient supplies to successfully support each sampling event.
- Paperwork checks – It is important to make sure that all field activities and measurements are properly recorded in the field. Therefore, prior to starting each field event, necessary paperwork such as field sheets, chain of custody record forms, etc. will be checked to ensure that sufficient amounts are available during the field event.

Field activity audits are held per the Central Valley Water Board's Procedures Manual to assess the sample collection methodologies, field measurement procedures, and record keeping of the field crew in order to ensure that the activities are being conducted as planned and as documented in this QAPP.

Post sampling event reviews will be conducted by the Contract Manager following each sampling event in order to ensure that all information is complete and any deviations from planned methodologies are documented. Activities include reviewing field measurement documentation in order to help ensure that all information is complete.

Laboratory data review will be conducted by the UC Davis QA Officer upon receipt of data from each lab. Data will be checked for completeness, accuracy, specified methods were used, and that all related QC data was provided with the sample analytical results.

Laboratory audits will include blind sample submission for a proficiency test for each sampling run. The results of the lab's analysis will be compared to the known analytes (e.g. lab blanks) or acceptable ranges (e.g. lab duplicates)

Assessment reports

Separate assessment reports will not be generated for readiness reviews, field activity audits, or post sampling event reviews.

Laboratory assessment information (data review and audit) will be included in the laboratory data sets.

Corrective action

If a problem arises, prompt action to correct the immediate problem and identify its root causes is imperative. Any related systematic problems must also be identified.

Problems regarding field data quality that may require corrective action will be documented in the field data sheets. Deficiencies that cannot be immediately corrected will be noted on the Central Valley Water Board field sheet and brought to the attention of the Contract Manager. The Contract Manager will coordinate with the Central Valley Water Board staff to correct the deficiencies. The results of the resolution of the discrepancy will be documented in writing on the field sheet and on a separate log that will be kept in the project file.

Individual laboratory data quality will be reviewed by the Contract Manager, Project Director and Project Manager for their respective labs. Deficiencies and corrective actions taken will be noted on the UC Davis Laboratory data sheet (Appendix 10) and Central Valley Water Board Bacteria Processing Worksheet (Appendix 9) as well as documented on the Excel spreadsheets to which the data will be transferred. Overall laboratory data quality will be reviewed by the UC Davis QA Officer.

The Contract Manager, Project Director and Project Manager have the authority to issue stop work orders to stop all sampling and analysis activities until the discrepancy can be resolved. .

C2, Element 21. Reports to Management

Interim and final reports

The Project Director will review draft reports to ensure the accuracy of data analysis and data interpretation. Raw data will be made available to data users per their request. The UCD QA Officer will report data to constituents after quality assurance has been reviewed. Every effort will be made to submit reports to the Central Valley Water Board staff in a fashion timely for their data uses.

Table 14 QA Management Reports

Type of Report	Frequency	Projected Delivery Dates(s)	Person(s) Responsible for Report Preparation	Report Recipients
Data report	Per field run	7/31/2009	UCD QA Officer	Contract Manager
Data report	Per field run	10/31/2009	UCD QA Officer	Contract Manager
Data report	Per field run	12/30/2009	UCD QA Officer	Contract Manager
Data report	Per field run	2/28/2009	UCD QA Officer	Contract Manager

Draft report	Annually	3/31/2010	UCD QA Officer	Contract Manager
Final report	Annually	5/31/2010	UCD QA Officer	Contract Manager

Quality assurance reports

Separate quality assurance reports will not be generated. Quality assurance information annotated on field and lab sheets will be included with the Data reports.

D1, Element 22. Data Review, Verification, and Validation Requirements

Data review, verification, and validation procedures help to ensure that project data will be reviewed in an objective and consistent manner. Data review is the in-house examination to ensure that the data have been recorded, transmitted, and processed correctly.

Responsibility for data review

The Contract Manager and UC Davis QA Officer will be responsible for data review. This includes checking that all technical criteria have been met, documenting any problems that are observed and, if possible, insuring that deficiencies noted in the data are corrected.

Checking for typical errors

In-house examination of the data produced from the proposed project will be conducted to check for typical types of errors. This includes checking to make sure that the data have been recorded, transmitted, and processed correctly. The kind of checks that will be made will include checking for data entry errors, transcription errors, transformation errors, calculation errors, and errors of data omission.

Checking against MQOs

Data generated by project activities will be reviewed against method quality objectives (MQOs). This will ensure that the data will be of acceptable quality and that it will be SWAMP-comparable with respect to minimum expected MQOs.

Checking against QA/QC

QA/QC requirements were developed and documented in B3, Element 12; B4, Element 13; B5, Element 14; B7, Element 16; and B8, Element 17 and the data will be checked against this information. Checks will include evaluation of field and laboratory duplicate results; and field and laboratory blank data pertinent to each method and analytical data set. This will ensure that the data will be SWAMP-comparable with respect to quality assurance and quality control procedures.

Checking lab data

Lab data consists of all information obtained during sample analysis. Initial review of laboratory data will be performed by the laboratory QA/QC Officer in accordance with the lab's internal data review procedures. However, once we receive the lab data then we will perform independent checks to ensure that it is complete, consistent, and meets the data management requirements of the data management section of this QAPP.

Data verification

Data verification is the process of evaluating the completeness, correctness, and conformance / compliance of a specific data set against the method, procedural, or contractual specifications. We will conduct data verification, as described in the Quality Control section, in order to ensure that it is SWAMP-comparable with respect to completeness, correctness, and conformance with minimum requirements.

Responsibility for data verification

The Contract Manager and the SWAMP Data Management Team will be responsible for verification of data going into the SWAMP IMS.

Data validation

Data validation is an analyte- and sample-specific process that evaluates the information after the verification process (i.e., determination of method, procedural, or contractual compliance) to determine analytical quality and any limitations. We will conduct data validation in order to ensure that the data is SWAMP-comparable with respect to its end use.

Responsibility for data validation

The Contract Manager and SWAMP Quality Assurance Team will be responsible for validation of data going into the SWAMP IMS.

Data separation

Data will be separated into three categories for use with making decisions based upon it. These categories are:

1. Data that meets all acceptance requirements
2. Data that has been determined to be unacceptable for use
3. Data that may be conditionally used and that is flagged as per US EPA specification

D2, Element 23. Verification and Validation Methods

Defining the methods for data verification and validation helps to ensure that project data are evaluated objectively and consistently. Information on these methods is provided below.

All data records for the proposed project will be checked visually and will be recorded as checked by the checker's initials as well as with the dates on which the records were checked. For data in the SWAMP IMS, the Central Valley Water Board will perform an independent re-check of at least 10% of these records as the validation methodology.

All of the laboratory's data will be checked as part of the verification methodology process. At least 10% of the laboratory's data will be independently checked as part of the validation methodology. For data in the SWAMP IMS, the Central Valley Water Board will perform independent re-checks of at least 10% of them as the validation methodology.

Any data that is discovered to be incorrect or missing during the verification or validation process will immediately be reported to the Contract Manager. If errors involve laboratory data then this information will also be reported to the UC Davis QA Officer. For data in the SWAMP IMS, the Central Valley Water Board and SWAMP DMT will be responsible for reporting and correcting any errors that are found in the data during the verification and validation process.

If there are any data quality problems we will try to identify whether the problem is a result of project design issues, sampling issues, analytical methodology issues, or QA/QC issues (from laboratory or non-laboratory sources). If the source of the problems can be traced to one or more of these basic activities then the person or people in charge of the areas where the issues

lie will be contacted and efforts will be made to immediately resolve the problem. If the issues are too broad or severe to be easily corrected then appropriate people involved will be assembled to discuss and try to resolve the issue(s) as a group. The Contract Manager has the final authority to resolve any issues that may be identified during the verification and validation process.

During the process of verification and validation for data in the SWAMP IMS, the methods that will be used are described in the Surface Water Ambient Monitoring Program Information Plan.

D3, Element 24. Reconciliation with User Requirements

Information from field data reports (including field activities, post sampling events, corrective actions, and audits), laboratory data reviews (including errors involving data entry, transcriptions, omissions, and calculations and laboratory audit reports), reviews of data versus MQOs, reviews against Quality Assurance and Quality Control (QA/QC) requirements, data verification reports, data validation reports, independent data checking reports, and error handling reports will be used to determine whether or not the project's objectives have been met.

Data from monitoring measurements will not be statistically analyzed. Descriptions of the data will be made with no extrapolation to more general cases.

Data from all monitoring measurements will be summarized in tables. In addition, data that show significant changes over time during the monitoring period will be plotted in graphs and charts. There are no known limitations that are inherent to the data to be collected for this study. Explanations will be provided for any data determined unacceptable for use or flagged for QA/QC concerns.

The proposed project will provide SWAMP-comparable data for the selected analytes described in Element 6. All data will be readily available to the public, and data for the analytes in Table 6.2 will be available from SWAMP's IMS and, subject to physical habitat limitations, the data generated will be useable for comparative purposes by other water monitoring projects within the various components of SWAMP.

The above evaluations will provide a comprehensive assessment of how well the project meets its objectives. No other evaluations will be used.

The Project Director and Contract Manager will be responsible for reporting project reconciliation. This will include measurements of how well the project objectives were met and

the degree to which the data is SWAMP-comparable.

This section describes how validated data will be evaluated to see if it answers the project objectives outlined in A5, Element 5.

APPENDICES

Appendix 1. List of sampling sites

Map ID	Station Number	Site Description	Land Use Representations	Historical Ranges (MPN/100 mL)	Latitude	Longitude
Sacramento Watershed						
1	A0275890 (DWR SWCMP)	Sacramento River below Red Bluff	A/E	980	40.1534	-122.1993
2	A0332000 (DWR SWCMP) 504ELD99W (SWAMP ID)	Elder Creek at Gerber	B/D	649	40.0509	-122.1666
3	A0290000 (DWR SWCMP) 504STNR24 (SWAMP ID)	Stony Creek at The Nature Conservancy (TNC)	E	>2420	39.6943	-121.9896
4	A0294710 (DWR SWCMP) 520CLSAKL (SWAMP ID)	Colusa Drain above Knights Landing	B	870	38.8121	-121.7741
5	A0219501 (DWR SWCMP) 519SACR16 (SWAMP ID)	Sacramento River below Knights Landing	A/D/E	548	38.7606	-121.6782
6	531PLA900 (SWAMP ID)	Dry Creek/ Cirby Confluence	D/E	210 - >2420	38.7335	-121.2885
7	544SAC007 (SWAMP ID)	American River at Discovery Park	D/E	187 - 1414	38.6017	-121.5027
Delta						
8	510TDNLHT (ILRP ID)	Toe Drain at Little Holland	B	170 - >2400	38.3491	-121.6450
San Joaquin Watershed						
9	532AMA002 (SWAMP ID)	Sutter Creek at Hwy 49	D/E	<1 - >2420	38.3926	-120.8013
10	544SAC002 (SWAMP ID)	Mokelumne River at New Hope Road	A/E	23 - >2420	38.2361	-121.4189
11	531SJC515 (SWAMP ID)	Bear Creek at Lower Sacramento Road*	B/D	15 - >2420	38.0428	-121.3214
12	536TUO208 (SWAMP ID)	Woods Creek at Mother Lode Fairgrounds*	D	84 - 1553	37.9778	-120.3903
13	535XLTABR (ILRP ID)	Lone Tree Creek at Bernnan Rd*	B/D	>1600	37.8255	-121.0159
14		Walthall Slough	C	NA	37.7692	-121.2891
15	535STC206	Dry Creek at La Loma Road	C	8 - >1600	37.6602	-120.8743
16	535STC501 (SWAMP ID)	Harding Drain at Carpenter Road*	B/D	<1 - >2420	37.4644	-121.0303

A – Integrator Site
B – Irrigated Agriculture
C – Confined Animal Feeding Operation
D – Community Development
E – Recreation
Sites not included in data collection

Appendix 2. Map of sampling sites



Appendix 3. Sampling event preparation

Sample Bottle Labeling

All samples will be pre-labeled before each sampling event to the extent practicable. Sample id numbers will correspond with the field sheets. Pre-labeling sample bottles simplifies field activities. Custom labels will be produced using blank water-proof labels. Using this approach will allow the stations and analytical constituent information to be entered into the computer program in advance, and printed as needed prior to each sampling event.

Labels shall be placed on the appropriate bottles in a dry environment; attempting to apply labels to sample bottles after filling will cause problems, as labels usually do not adhere to wet bottles. The labels shall be applied to the bottles rather than to the caps. Field labels shall contain the following information:

Sampler initials, year, month, date – sample id, parameter identification.

Parameter identifications are as follows:

BAC	Total coliform/ <i>E. coli</i>
O157	<i>E. coli</i> O157:H7
DNA	<i>Bacteroidales</i>
SAL	<i>Salmonella</i>

Example:

CLG090505-10BAC
CLG090505-10O157
CLG090505-10DNA
CLG090505-10SAL

Appendix 4. Field Protocols

Field crews (2 persons per crew, minimum) will only be mobilized for sampling when weather conditions and flow conditions are considered to be safe. For safety reasons, sampling will occur during daylight hours. A sampling event should proceed in the following manner:

1. Before leaving the sampling crew base of operations, notify laboratory, confirm number and type of sample bottles as well as the complete equipment list.
2. Proceed to the first sampling station.
3. Fill-out the general information on the field log sheets (Appendices 6&7).
4. Take field measurements and observations, and record on the field log sheet (Appendix 7).
5. Take the samples indicated on the field log sheet in the manner described in this plan. Place bottles in the coolers with ice. Double check against the log sheet that all appropriate bottles were filled.
6. Repeat the procedures in steps 3, 4, and 5 for each of the remaining sampling stations.
7. Complete the chain of custody forms (Appendices 8&9) using the field notes.
8. After collection is completed, deliver the samples to Atwill laboratory at the end of the field run.

Appendix 5. Sample collection

Water Sample Collection

All water samples will be collected as grab samples, using aseptic technique. At most stations, grab samples will be collected at approximately six feet from the bank, using sampling poles, by direct submersion of the sample bottle depth. This is the preferred method for grab sample collection; however, due to sampling station configurations and safety concerns, direct filling of sample bottles is not always feasible. Sampling station configuration will dictate grab sample collection technique. Grab samples will be collected directly into the appropriate bottles (containing the required preservations). The grab sample technique that may be employed is described below.

Direct Submersion:

Where practical, all grab samples will be collected by direct submersion to mid-stream, mid-depth using the following procedures.

1. Wear clean powder-free nitrile gloves when handling bottles and caps. Change gloves if soiled or if the potential for cross-contamination occurs from handling sampling materials or samples;
2. Submerge bottle to mid-stream/mid-depth, remove lid, let bottle fill, and replace lid. Place sample on ice;
3. Collect remaining samples including control samples, if needed, using the same protocols described above;
4. Fill out COC form, note sample collection on field form, and deliver to Atwill lab.

Field Measurements and Observations

Field measurements will be taken and observations made at each sampling station before a sample is collected. Field measurements will include pH, temperature, dissolved oxygen, electrical conductivity and turbidity. Field measurements will be taken at approximately six feet from the bank. All field measurement results and comments on field observations will be recorded in the field log in Appendices 6&7.

If at any time the collection of field measurements appears unsafe, an alternate site within 100 yards may be used, or the sample will not be collected. Sample site conditions will be noted on the field sheets, and photos will be taken of the site.

In addition to field measurements, observations will be made at each sampling station. Observations will include color, odor, floating materials, presence of wildlife, as well as observations of contact and non-contact recreation. All comments on field observations will be recorded in the field log presented in Appendices 6&7.

Chain-of-Custody

Chain-of-custody (COC) forms will be filled out for all samples submitted to the Atwill laboratory. Sample data, sample location, sample collection crew names, and analysis requested shall be noted on each COC. See Appendices 8&9 for blank COC forms.

Transport to Lab

Samples will be stored in coolers with ice and delivered to UC Davis at the address provided in the field protocols section of this plan.

The collector will also be careful to not touch the inside of the sample bottle at anytime. If the inside of the sample bottle is accidentally touched another sample bottle will be used. This is the preferred method for grab sample collection, and shall be adhered to as long as the safety of the sampling personnel is not jeopardized by doing so. Modifications are to be made only as necessary, and clean sampling techniques are always to be followed. After collection the samples will be immediately placed on ice in a cooler for transport to UC Davis laboratories. All samples to be analyzed by the Atwill and Wuertz laboratories will be delivered to the Atwill Laboratory at the end of the field run. Samples for total coliform and *E.coli* will then be delivered to the Central Valley Water Board laboratory. Analysis of the samples will be initiated within 24 hours of the collection time of the first sample. All specimen and test data will be captured in the Laboratory Information System. Control samples will be collected at the same time and also immediately placed on ice. The proper COC form (See Appendices 8&9) will be filled out and signed by the appropriate lab personnel prior to releasing the samples to them.

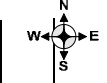
Clean Sampling Techniques

Samples will be collected using “clean sampling techniques” to minimize the possibility of sample contamination. For this program, clean techniques must be employed whenever handling bottles, lids, or intermediate containers. Clean sampling techniques are summarized below:

- Samples are collected only into new, clean, laboratory provided sample bottles.
- Wearing clean powder-free nitrile gloves at all times are required on sampling crews.
- Clean, powder-free nitrile gloves are changed whenever something not known to be clean has been touched.
- Clean techniques must be employed whenever handling grab sample or intermediate bottles.
- To reduce the potential for contamination, sample collection personnel must adhere to the following rules while collecting samples:
 - No smoking.
 - Never sample near a running vehicle. Do not park vehicles in immediate sample collection area, even non-running vehicles.

- During wet weather events avoid allowing rainwater to drip from rain gear or any other surface into sample bottles.
- Do not eat or drink during sample collection.
- Do not breathe, sneeze or cough in the direction of an open sample bottle.

Appendix 6. Field Sheet (SWAMP)

SWAMP Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ										Entered in d-base (initial/date)		Pg of Pgs	
*StationID: 532AMA002 (Sutter Creek at Highway 49)				*Date (mm/dd/yyyy): 5/26/2009				*Group: SWAMP		*Agency: Central Valley Water Board			
*Funding: 156-01				ArrivalTime:		DepartureTime:		*SampleTime (1st sample):		*Protocol:			
*Personnel: CLG/MTS				*Purpose (circle all that apply): WaterChem WaterTox FieldObs FieldMeasure						*PurposeFailure:			
*Location: Bank Thalweg Midchannel OpenWater				*GPS/DGPS		Lat (dd.ddddd)		Long (ddd.ddddd)		OCCUPATION METHOD: Walk-in Bridge R/V Other			
GPS Device:				Target:		38.3926		-120.8013		STARTING BANK (facing downstream): LB / RB / NA			
Datum: NAD83		Accuracy (ft / m):		*Actual:		38.3926		-120.8015		Point of Sample (if Integrated, then -88 in dbase)			
Field Observations (SampleType = FieldObs)						WADEABILITY: Y / N / Unk		BEAUFORT SCALE (see attachment):		DISTANCE FROM BANK (m):		STREAM WIDTH (m):	
SITE ODOR: None, Sulfides, Sewage, Petroleum, Mixed, Other												WATER DEPTH (m):	
SKY CODE: Clear, Partly Cloudy, Overcast, Fog						WIND DIRECTION (from):				HYDROMODIFICATION: None, Bridge, Pipes, ConcreteChannel, GradeControl, Culvert, AerialZipline, Other sample): US / DS / WI / NA			
OTHERPRESENCE: Vascular, Nonvascular, Oily Sheen, Foam, Trash, Other										PHOTOS (RB & LB assigned when facing downstream; RENAME to StationCode_yyyy_mm_dd_uniquecode):		1: (RB / LB / BB / US / DS / ##)	
DOMINANTSUBSTRATE: Bedrock, Concrete, Cobble, Gravel, Sand, Mud, Unk, Other												2: (RB / LB / BB / US / DS / ##)	
WATERCLARITY: Clear (see bottom), Cloudy (>4" vis), Murky (<4" vis)						PRECIPITATION: None, Fog, Drizzle, Rain, Snow						532AMA002_2009	
WATERODOR: None, Sulfides, Sewage, Petroleum, Mixed, Other						PRECIPITATION (last 24 hrs): Unknown, <1", >1", None						532AMA002_2009	
WATERCOLOR: Colorless, Green, Yellow, Brown												3: (RB / LB / BB / US / DS / ##)	
OBSERVED FLOW: NA, Dry Waterbody Bed, No Obs Flow, Isolated Pool, Trickle (<0.1cfs), 0.1-1cfs, 1-5cfs, 5-20cfs, 20-50cfs, 50-200cfs, >200cfs												532AMA002_2009	
Field Measurements (SampleType = FieldMeasure; Method = Field)													
	DepthCollec (m)	Velocity (fps)	Air Temp (°C)	Water Temp (°C)	pH	O ₂ (mg/L)	O ₂ (%)	Specific Conductivity (uS/cm)	Salinity (ppt)	Turbidity (ntu)	Stage Ht (units)		
SUBSURF/MID/BOTTOM/REP													
SUBSURF/MID/BOTTOM/REP													
SUBSURF/MID/BOTTOM/REP													
Instrument:													
Calib. Date:													
Samples Taken (# of containers filled) - Method=Water_Grab						Field Dup YES / NO: (SampleType = Grab / Integrated; LABEL_ID = FieldQA; create collection record upon data entry)							
SAMPLE TYPE: Grab / Integrated						Indiv bottle (by hand, by pole, by bucket); Teflon tubing; Kemmer; Pole & Beaker; Other							
	DepthCollec (m)	Inorganics	Bacteria	Chl a	TSS / SSC	TOC / DOC	Total Hg	Dissolved Mercury	Total Metals	Dissolved Metals	Organics	Toxicity	VOAs

Sub/Surface													
Sub/Surface													
COMMENTS:													

Appendix 7. Field Sheet (Central Valley Regional Board)

Source ID Study
SJRWU Monitoring Data Sheet
(revised 2/26/08)

*Note YSI: #1=19869; #2=19859; #3=18243; #4=18471

(Note: Nutrients twice a month March -Aug)

Sampler: _____
Project: Source ID

Date: _____
Vehicle # _____

YSI METER # _____

Sample ID - Code		Site Code	Site Description	Sampling Type	YSI					Dry	Lab SC	Pic #
					Time	Temp (C)	Field SC	DO mg/L	pH			
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								
				G								

Time	Calibrations	Initials	pH	pH MV			DO		Conductivity		Recal	Lab EC
			7.0	4.0	7.0	10.0	Charge	Gain	Sp. Cond	Cal Const.	Y/N	
	Beginning								/			
	End								/			
	ph slope 165-180 MV		+/- 0.2	+177	+/-50	-177	50 +/-25	-0.7to1.4	+/- 20	5.0 +/- .45		

Bac - Total coliform / *E. coli*

O157 - *E. coli* O157:H7

ID - *Bacteroidales*

S - *Salmonella*

G-Grab Sample

Sample Processing

Initials / Date/Time

Total coliform / *E. coli* _____

Tray Reading _____

Data transfer to UCD _____

NOTES: WEATHER CONDITIONS, WATER LEVELS

Appendix 8. Form for Chain of Custody (UC Davis)

Program:				Project Name: Central Valley Bacterial source		Preservative	Sampling method		ANALYSIS REQUIRED	
Sampler (Name):				Sampler (Signature):			INTEGRATED GRAB	GRAB	E.coli O157	Bacterial source
SAMPLE INFORMATION						CONTAINER				
IDENTIFICATION	LOCATION	DATE	TIME	TYPE	NO.					
				1L		ice				
				1L		ice				
SPECIAL INSTRUCTIONS / SUSPECTED CONSTITUENTS:										
RELINQUISHED BY (Signature) DATE/TIME				RECEIVED BY (Signature)				RECEIVED BY (Signature)		
RELINQUISHED BY (Signature) DATE/TIME				RECEIVED BY (Signature)				RECEIVED BY (Signature)		

Appendix 9. Bacteria Processing Worksheet (Central Valley Regional Board)

Run: Source ID Study

Sample Processing Date:

Sample ID Number:																	
Site Code:																	
Yellow +	# Small Wells																
	# Large Wells																
	Empty Wells																
	MPN																
Yellow + Fluorescence (+)	# Small Wells																
	# Large Wells																
	False Positives																
	MPN																
Start Temp/Time		End Temp/Time															
	FIELD DUPLICATES								LAB DUPLICATES								
	Normal Sample #								Normal Sample #								
	Duplicate Sample #								Duplicate Sample #								
		MPN	95% CI							MPN	95% CI						
			Lower	Upper							Lower	Upper					
TOTAL COLIFORM	Normal								Normal								
	Duplicate								Duplicate								
	Mean			Pass	Needs Review				Mean			Pass	Needs Review				
E. COLI	Normal								Normal								
	Duplicate								Duplicate								
	Mean			Pass	Needs Review				Mean			Pass	Needs Review				
BLANKS	Travel Sample #14			Pass	Needs Review				Lab Sample #13			Pass	Needs Review				

Mean = Mean of Normal and Duplicate, which is then compared to the individual corresponding CI's to determine acceptability of data

Sampler Signature / Date / Time Arrived in Lab:

Placed in Incubator By / Date / Time:

Trays Read By:

Processor / Date / Time:

Pulled from Incubator By / Date / Time:

Entered into database:

NOTES:

[illegible]

Appendix 11. Protocol 1. (Atwill lab). Detection of E.coli O157 from waters by Enrichment-ImmunoMagneticSeparation

A qualitative Enrichment-ImmunoMagnetic Separation method will be used for the determination of presence/absence of *E. coli* O157:H7 in waters. The following sections outline the procedures of this method.

Enrichment

Using an automatic pipet and a 50mL serological tip (Fisher 13-676-10Q) aspirate 100mL of environmental water into a sterile 250mL Pyrex graduated Erlenmeyer flask with wide mouth, screw cap (Fisher 10-041-6A). Aseptically add three grams Tryptic Soy Broth (BD brand, Fisher DF0370-17-3) to the flask and agitate until medium is dissolved. Place the flask in the center of the shaking incubator (Multitron II AJ12¹⁰). Set the shaking incubator "F program" to 200 RPM at 25°C for 2 hours (to set the incubator scroll through the three settings using the F key and arrows and make sure to turn each setting on by pushing the start button). After completion of the "F program", set a new "F program" to 200 RPM at 42°C for 8 hours. Set the "F" program" to 6°C and make sure to turn off the RPM and Timer settings. After incubation is complete remove the flasks from the incubator.

Immuno Magnetic Separation

Just prior to removing the flask from the incubator, prepare for use of the Dynal BeadRetriever (Invitrogen 159-50). Insert sterile Dynal tips (Invitrogen 159-51) into Dynal machine. Place sterile tubes (Invitrogen 159-51) into Dynal tube rack (Invitrogen 159-52). Add 500uL wash buffer¹ to wells 1 & 2, add 1000uL wash buffer to wells 3 & 4, and finally add 100uL wash buffer to well 5. From the flask, add 500uL of sample to well 1 & 2. Aseptically add 10uL of anti-O157 DynalBeads to well 1 & 2 (Invitrogen 710-04). Place the tube tray in the Dynal BeadRetriever, scroll with the arrow key to set machine to EPEC/VTEC, and then push the start button. After the machine has finished plate 50uL from well 5 onto CT-SMAC II agar plates and 50uL from well 5 onto Rainbow agar plates; streak for isolation.

Appendix 12. Protocol 2. (Atwill lab). PCR for O157:H7 determination

For O157 positive samples, a specific PCR will be used for determination of groups of O157. The following sections outline the PCR procedures.

Day 1:

- 1) Samples from frozen microbanks are streaked for isolation on a plate of LB Agar. Incubate for 24 hours at 37°C.

Day 2:

- 2) Place 1.5mL microcentrifuge tubes, DNase free water, light blue 1uL disposable loops, pipet, and pipet tips into hood and UV for 15 minutes.
- 3) Aliquot 100uL of DNase free water into each 1.5mL microcentrifuge tube.
- 4) Scrape a loopful of colonies off of each LB agar plate with a light blue 1uL disposable loop and inoculate a 1.5mL microcentrifuge tube.
Try to remove as much of the sample off of the loop as possible, by swirling the loop inside the DNase water.
- 5) Boil the sample tube at 100°C for 20 minutes.
- 6) Centrifuge the sample for 10 minutes at 5000 rpm.
- 7) If there is no pellet, start from Step 1 of Day 2.
- 8) If there is a pellet present, aspirate the supernatant without pellet disruption and transfer the supernatant into a new 1.5mL microcentrifuge tube.

PCR

- 1) Pre-sterilize all PCR equipment using UV-light
- 2) Remove PCR reagents from the Freezer, keep chilled in a Labtop cooler -20°C
- 3) Prepare primer by adding 10 DEPC-Treated water to 1 Primer Concentrate (Proligo Oligo 3 O.D. 100uMol)
 - a. Forward Primer: 3' CGG ACA TCC ATG TGA TAT GG 5'
 - b. Reverse Primer: 5' TTG CCT ATG TAC AGC TAA TCC 3'
- 4) Prepare dNTP's by adding 10 DEPC-Treated water to 1 dNTP
- 5) Prepare "Master Mix" by adding 32.25uL DEPC-Treated water (x # samples being PCR) + 5uL Buffer (x # samples) + 3uL MgCl₂ (x # samples) + 1uL dNTA (x # samples) + 1uL dNTC (x # samples) + 1uL dNTG (x # samples) + 1uL dNTT (x # samples) + 2uL Forward Primer (x # samples) + 2uL Reverse Primer (x # samples) + 0.25uL AmpliTaq (x # samples) into a Microcentrifuge tube.
- 6) Centrifuge "Master Mix" at 1000 RMP for 1 minute
- 7) Dispense 48.5uL "Master Mix" into 0.2mL PCR tube
- 8) Add 1.5uL DNA to 0.2mL PRC tube
- 9) Place tube into Master Cycler with the following parameters
 - a. Initial denaturation 95°C for 1min

- b. 30 cycles of denaturation at 94°C for 15 seconds, annealing 55°C for 15 seconds, and extension at 72°C for 1 minute
- c. Final Extension of 72°C for 1 minute
- d. Hold sample at 4°C until removed from thermocycler.

Gel Electrophoresis

- 10) Run PCR product on a 2% Agarose gel
- 11) Use Invitrogen Low Mass Ladder
- 12) Stain with ethidium bromide
- 13) View gel under UV-Transillumination
- 14) PCR product should yield a 259bp amplicon

Appendix 13, Protocol 3 (Atwill Lab) Method for Detection of *Salmonella* from Waters

A multiple tube method is used for detection of *Salmonella* from waters. The following sections outline the procedures of this method.

Using a vacuum filtering system, 200ml (×3 replicates), 20ml (×3 replicates), 2ml (×3 replicates), and 0.2ml (×3 replicates) waters are filtered through 0.45µm membrane filters. Filters are folded and placed into wells of 12-well chamber reservoirs (each well contain 3ml buffered peptone water (BPW)), and pre-enriched by incubating 24 hours at 37°C. Ten microliters of the pre-enrichment solution were then transferred into wells of 96-well plate each contains 1.0 ml of Rappaport-Vassiliadis (RV) and enriched by incubating for 24 hours at 42°C.

Five microliters of the enrichment solution from each well is streaked as a lane on rectangle Xylose Lysine Deoxycholate (XLD) agar plates. Lanes with black colonies are suspect positive *Salmonella* reaction which is confirmed by biochemical tests (Triple Sugar Iron Agar, Urea Agar, and Lysine Iron Agar). For each sample, the numbers of positive reaction lanes of each volume filtered are recorded. The concentrations of *Salmonella* in waters are calculated as Most Probable Number (MPN) using the shareware software package MPN calculator (Mike Curiale).

Appendix 14. Protocol 4. (Wuertz Lab): Membrane Filtration using the MO Bio UltraClean Water DNA Isolation Kit

1. Spike water sample with 20 µL *Acinetobacter* stock solution. Mix thoroughly.
2. Collect 5 mL sample in plastic container (e.g. 15 mL centrifuge tubes). Add "Feed" to sample label.
3. Record spiked volume and later filtered volume either on label or COC form.
4. Filter 500 mL of the water samples using Water filters provided. Use the filter adapter provided in your filtration assembly. The volume of water you filter depends on the microbial load and turbidity of the water sample. Filter at least 250 mL in case of increased filtration times
5. Aseptically remove the filter membrane. The 100 ml upper portion of the filter cup is easily removed from the catch reservoir by snapping it off.
6. Use sterile forceps to pick up the white filter membrane.
7. Insert the filter into a Water Bead Tube.
8. Add 4 ml of Bead Solution to the Water Bead Tube.
9. Vortex for 30 seconds.
10. Store tubes at least at -30 °C prior to further treatment

Appendix 15. Protocol 5 (Wuertz lab) Bacteroidales

Quantitative PCR will be used for to determine filtration recovery of bacteria based on a surrogate *Acinetobacter* strain and for the detection of Bacteroidales. The following section will outline the general treatment used for TaqMan® analysis, as well as specific assays used in detection. For each run of Bacteroidales assays, both negative and positive controls are included. Should failures occur because these controls do not perform as expected the runs will be repeated. The following is basic protocol that is to be used for any TaqMan® assay, regardless of target sequence or organism type.

Protocol

1. Thaw samples to be analyzed, and mix well with a vortex. Extract nucleic acid using the Qiagen QiaAmp Viral RNA Mini Kit (small-scale extraction) according to manufacturer's instructions or the large-scale extraction protocol outlined previously.
2. Prepare the appropriate dilutions of sample RNA using RNase, DNase free molecular grade water. The dilutions for the extractions should not be made more than a half an hour ahead of time, and should remain covered at 4°C until used.
3. Determine the total number of reactions needed (all dilutions in duplicate, plus a negative control, and a positive control when appropriate) and prepare a master mix appropriate for the microbe of interest. The negative control should be composed of the same water used to make the master mix. The master mix should always be made in a DNA/RNA free zone.
4. Load the master mix into a 96 well plate, and cover with foil adhesive cover before leaving the DNA/RNA free zone.
5. Load extract dilutions into the 96 well plate, which contains the previously made mastermix. Cover the plate with an optical adhesive cover, making sure that the adhesive cover is sealed across each well and at all sides of the cover. Pulse centrifuge to collect all liquid to the bottom of each well, and to remove any bubbles that may have been produced during the nucleic acid transfer.
6. Run the appropriate thermocycling profile for the microbe of interest.
 - 1) Use Ct values to calculate total concentration of the microbe of interest per reaction by applying a standard curve².
 - 2) Calculate the corresponding concentration of the microbe of interest in the sample volume added to the TaqMan® reaction. Apply the appropriate equation³ to determine

² **Standard curve**

Plasmids, containing the specific marker sequence are quantified by spectrophotometry to generate standard curves in triplicate by real-time TaqMan PCR for each assay. These standard curve allow relative calculation of target concentrations.

³ **Calculation of Target Quantity in Sample Retentate**

When a positive signal is received from TaqMan qPCR, the concentration of the target organism in the original water sample was calculated with following equation:

the original concentration of the microbe in the environmental water sample.

- 3) If no target is detected in a TaqMan® reaction, determine the detection limit for the microbe of interest⁴.

$$Concentration = \left(\frac{T}{V_T} \right) \left(\frac{V_{el}}{V_{RF,ex}} \right) \left(\frac{V_{RF}}{(V_s)(R_{filtration})(E_{ex,FLS})} \right)$$

The concentration calculation (gene copies/mL) utilized the recovery of the surrogate *Acinetobacter* for *Bacteroidales* in order to predict the amount of target lost during the filtration process. All values remain the same as for detection limit calculation but the analytical detection limit (A_{LOD}) is replaced by T, the gene copy number of target organisms for the undiluted sample from the TaqMan reaction, as determined by the TaqMan standard curve.

⁴ Calculation of sample detection limits (SLOD)

The S_{LOD} (gene copies/mL) is calculated for each original volume of sample according to:

$$S_{LOD} = (A_{LOD}) \left(\frac{I}{V_T} \right) \left(\frac{V_{el}}{V_{RF,ex}} \right) \left(\frac{V_{RF}}{(V_s)(R_{filtration})(E_{ex,FLS})} \right)$$

Where V_{RF} is the volume of final concentrated retentate (mL), $V_{RF,ex}$ the volume of retentate that was extracted (mL), V_{el} of eluate from nucleic acid extraction (mL), V_T volume of nucleic acid template added to the PCR reaction (mL). The volume of the original water sample is V_s (mL). Inhibition factor I represents the dilution necessary to produce a positive PCR result and is expressed as the inverse of the dilution factor.

The overall recovery is assessed by measurement of the surrogate *Acinetobacter*, is represented by $R_{filtration}$ (0-1), while $E_{ex,FLS}$ (0-1) accounts for the nucleic acid extraction efficiency. A_{LOD} , the assay limit of detection, was previously obtained specifically for each target for pure water by following the general approach outlined in the US EPA method 40 CFR 136, Appendix B.

Appendix 16. Total Coliform and *E. coli* Sample Processing (Central Valley Regional Board Lab)

- Equipment Preparation
 - Sealer – 15 minutes to warm up
 - Incubator – 90 minute minimum to warm up
 - Temperature check (35°C)
- Meeting the field crew
 - Sample packing in the cooler
 - Verify correct packing
 - Annotate on field sheet and processing worksheet
 - CoC Verification
 - Sampler signature, date, time
 - Processor signature, date, time
- Procedure
 - Gather supplies

Gloves	Eyewear	Lab Coat	DI Water
Quantitrays	Quantitray	Snap Packs	Glass Beaker
	Labels		
Lab blank	Sink Rack	Full sample	Antifoam
		bottles	Solution
Bacteria Processing Worksheet		Spray Bottle filled with alcohol	
 - Work area/sample bottle prep
 - Spray work area with alcohol
 - Triple rinse outside of sample bottles (inc. lab splits) with DI poured from beaker
 - Dry bottles and line them up on the work area in numerical order
 - For consistency purposes, this action initiates the two hour processing time window.
 - Split duplicate samples
 - This is done first so that all samples are treated the same
 - Place 250 ml sample and corresponding 100 ml bottles in the work area.
 - Remove the shrink bands from the two 100 ml bottles.
 - Remove the bottle caps once the alcohol has evaporated
 - Gently invert the 200 ml bottle at least ten times to resuspend any matter that settled.
 - Pour samples into the 100 ml bottles, alternating bottles with equal amounts of sample in approximately 50 ml increments, and swirl the 200 ml bottle between pours.
 - Line up the 100 ml bottles with the rest of the sample set.

- Sample processing
 - Ensure correct volume
 - Pour off into cap, if necessary
 - Infuse samples
 - Loosen but do not remove the cap
 - Add snap pack contents and 2 drops antifoam solution to sample
 - Mix sample (gently swirl)
 - Watch for normal color changes vs. abnormal, i.e., blue flash
 - Repeat steps for each sample
 - Mix until snap pack contents from all bottles are completely dissolved.
- Transfer samples to tray after all samples are completely mixed
 - Place pre-filled labels on the back side of trays
 - Swirl the bottle, loosen but do not remove cap
 - Hold the Quantitray at a 45° angle, keeping the wells facing upwards
 - Remove the cap from the bottle and slowly pour the sample down the foil side of the tray
 - Tap out bubbles
 - Place tray on sealer insert and push into the sealer until the sealer pulls the insert
 - Do not push the insert once the sealer catches it
 - Do not pull the insert out the back until the sealer releases it
 - Remove the insert and tray from the rear of the sealer
- Place samples in the incubator after all trays are sealed
 - Note incubator temperature prior to opening the door on the bacteria processing worksheet
 - Place trays in incubator
 - Wells face down
 - Evenly distributed
 - Open door minimum amount of time
 - Annotate a Post it with the date, time trays were put in the incubator, and processor initials and attach it to the incubator door.
 - Keep CoC with the incubator
- Clean up
 - Disinfect all work surfaces with alcohol
 - Rinse sample bottles with tap water and dispose of bottles in the garage recycling bin

Appendix 17. Total Coliform and *E. coli* Sample Tray Reading (Central Valley Regional Board Lab)

- Incubation time
 - 18-22 hour pull time
 - For consistency of sample handling, pull at 18 hours
- Supplies

Gloves	Lab Coat	Comparator
UV Goggles	UV Light	Sharpie Marker
(UVEX)		
UV Viewing Cabinet		Bacteria Results Worksheet
- Record incubator temperature on the Bacteria Processing Worksheet prior to opening the incubator door
- Reading wells
 - Total coliform
 - Yellow wells in normal light
 - First set of lines (I)
 - Write number of positive wells on the upper right corner of the set of large wells and set of small wells.
 - *E. coli*
 - Fluorescent wells in UV light
 - Use viewing box for consistent UV intensity
 - Use Comparator to ensure positive wells fluoresce
 - Second set of lines (-)
 - Write number of positive wells on the lower right corners of the set of large wells and set of small wells
 - Items to watch for:
 - More positive fluorescent wells than positive yellow wells
 - Empty wells
 - All positive wells are marked
- Record results on Bacteria Processing Worksheet:
 - Total positive yellow and fluorescent wells
 - Empty wells
 - False positive results
 - All CoC/QA information
- Duplicate Samples QA Verification
 - Run the MPN generator program (Can be downloaded from <http://www.idexx.com/water/quantitray>)
 - Passing QA
 - Mean MPN falls within both confidence limits
 - Transfer QA data to the log sheet in the Bacteria QA binder
 - QA doesn't pass
 - Mean MPN falls outside one or both confidence limits

- Verify sample numbers
 - Verify data entry
 - If needed, contact the program manager to verify tray counts
 - Transfer QA data to the log sheet
- Bacteria Processing Worksheet / Chain of Custody form
 - Items to watch for
 - Number of wells recorded does not exceed 48 small wells and 49 large wells
 - All items at the bottom of the bacteria processing worksheet are filled in
 - Forward to appropriate person to be entered to database
- Tray Disposal
 - Biohazard cans
 - Monthly disposal submitted to Sacramento County Sanitary District

Appendix 18. Signed Signature Pages

A1, Element 1. Title and Approval Sheet

Program Title Central Valley Bacteria Source Identification Study

Contracted Organization University of California, Davis
Department of Health and Reproduction
Department of Civil and Environmental Engineering
1 Shields Drive
Davis, CA 95618

Lead Organization Central Valley Regional Water Quality Control Board
San Joaquin River Watershed Unit
11020 Sun Center Drive, #200
Rancho Cordova, CA 95670

Primary Contact Catherine Gill, Environmental Scientist
Phone Number: 916 464-4714
Email Address: cgill@waterbaords.ca.gov

Effective Date May 2009

Moss Landing Marine Laboratories, SWAMP Program

Quality Assurance Officer, Quality Assurance Research Group: Beverly van Buuren

Signature:  Date: 05/05/09

University of California, Davis

Project Director: Rob Atwill

Signature:  Date: 5/22/09

Project Manager: Stefan Wuertz

Signature: _____ Date: _____

A1, Element 1. Title and Approval Sheet

Program Title Central Valley Bacteria Source Identification Study

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Department of Health and Reproduction
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1 Shields Drive
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Moss Landing Marine Laboratories, SWAMP Program

Quality Assurance Officer, Quality Assurance Research Group: Beverly van Buuren


Signature: _____ Date: _____

University of California, Davis

Project Director: Rob Atwill

Signature: _____ Date: _____

Project Manager: Stefan Wuertz

Signature:  _____ Date: 06/25/2009

QA Officer: Xunde Li

Signature: Xunde Li Date: 6/23/09

Central Valley Regional Water Quality Control Board

Contract Manager: Catherine Gill

Signature: Catherine Gill Date: 7/13/09

QA Officer: Leticia Valadez

Signature: Leticia Valadez Date: 6/22/2009

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